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BIOCHEMICAL GENETICS OF THE INTRODUCED MARINE FANWORM
SABELLA SPALLANZANII

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CONTENTS

1. SUMMARY

Three Australian (Cockburn Sound, Western Australia; Adelaide, South Australia; Port Phillip Bay, Victoria) and four European (Roscoff, Atlantic; Alicante, Carteau, and Marseille, all from the Mediterranean Sea) populations of *Sabella spallanzanii* were examined for variation at 23 allozyme loci. Levels of genetic variation were high, with average heterozygosities per locus ranging from 0.20 to 0.21 for the Australian collections, and from 0.23 to 0.27 for the European collections. The introduction to Australia had therefore been accompanied by the loss of about 18% of the original variation.

The four European collections showed limited, but significant, differentiation among themselves. Ten of the 18 variable loci showed significant heterogeneity. Much of the heterogeneity reflected differences between the Roscoff (Atlantic) collection and the three Mediterranean collections, although there were also significant differences between the Alicante and Marseille/Carteau collections. The Roscoff collection was less variable, in terms of both heterozygosity and mean numbers of alleles, than the Mediterranean collections, suggesting that the species was once endemic to the Mediterranean but in the course of expanding its range to the Atlantic variation was lost through bottlenecks in population size.

The Australian collections were more closely related to the Mediterranean collections than to the Roscoff collection, indicating that a Mediterranean origin was more likely than an Atlantic origin.

The three Australian collections were genetically very closely related to one another, with no statistically significant differences at any of the 15 variable loci. This suggests either the likelihood of a single European introduction to one port, with a subsequent spread to other locations, or multiple introductions from a single European source.

Finally, the study confirms, from the very high genetic similarity of the Australian to the European collections of *S. spallanzanii* (mean Nei distance = 0.051), and the very clear genetic separation of *S. pavonina* (mean Nei distance = 0.996), that the Australian collections really do consist of *S. spallanzanii* and not an undescribed native species.

2.   INTRODUCTION

Sabella (Spirographis) spallanzanii is a marine fanworm. It is a member of the family Sabellidae, class Polychaeta, phylum Annelida. Most publications referring to this species refer to it as *Spirographis spallanzanii* Viviani (1805), but it appears to have been recognised earlier as *Sabella spallanzanii* Gmelin 1791. Ewer (1946) synonymised the genera *Spirographis* and *Sabella*, and recently Perkins and Knight-Jones (1991) synonymised *Spirographis spallanzanii* Viviani with *Sabella penicillus* Linnaeus, but suggested suppressing *penicillus* and other synonyms to stabilize *spallanzanii*. That is the nomenclature adopted herein.

This species is widespread in the Mediterranean, being recorded from the coasts of, at least, Spain, France, Italy, Turkey and Morocco. It is also found on eastern Atlantic coasts, from Morocco (Bitar, 1987), and from Portugal (Zibrowius, pers. comm.) to north-west France, and in the Azores. There is a record of an isolated, presumably introduced, population in Rio de Janeiro (P. Knight-Jones, pers. comm., referred to in Carey and Watson, 1992). It is found attached to the substrate in waters from 1 to 30 m in depth, and is particularly prevalent in harbours, where it can rapidly colonise man-made structures (Koechlin, 1977; Clapin and Evans, 1995).

Giangrande and Petraroli (1994) studied the species from the Ionian Sea and described it as a protandric sequential hermaphrodite, and with females larger than males. It is a broadcast spawner, with strings of mucus containing eggs or sperm ejected into the water from the coelomic cavity (Gravier, 1923). Like all sabellids (Rouse and Fitzhugh, 1994), it has non-feeding (lecithotrophic) larvae.

Sabella spallanzanii was first reported from Australian waters in the Geelong arm of Port Phillip Bay, Victoria (Carey and Watson, 1992). Although it had not been seen in the Bay prior to the early 1980's, by 1991 it had become common in Corio Bay, being described as the "visually dominant organism of the muddy bottom habitat" and releasing "masses of green-coloured eggs" in late summer (Carey and Watson, 1992). Scallop fishers in the Geelong arm of Port Phillip Bay first noticed the fanworm in their catches in 1993, and since then catches of the fanworm have increased, clogging dredges and increasing the time needed to sort scallops. The fanworm has also hindered the snapper fishery through interference with longlines.

In September 1994, dense beds of sabellid worms were found by Clapin and Greenway in Cockburn Sound (Western Australia). Inspection of aerial photographs suggested that fanworm patches could be identified in photographs dating back to 1983. This was supported by anecdotal evidence and by the photographing of what appeared to be specimens of *S. spallanzanii* in 1985 (Clapin and Evans, 1995).

In March 1995, the two Albany (Western Australia) ports were surveyed, and both contained *S. spallanzanii*. Examination of sabellids from the Western Australian museum revealed three apparent specimens of *S. spallanzanii*,

collected from Albany in 1965, 1978 and 1979. Thus this fanworm may have been present in this region for at least 30 years (Clapin and Evans, 1995).

In 1995, large numbers of *S. spallanzanii* were found in the vicinity of Adelaide, but records held by the South Australian museum indicate that the species may have been present since at least the mid-1970's (Rainer, pers. com.).

Once established in a suitable habitat, the species can rapidly proliferate, reaching high densities. It may directly compete with native species for space and food, and indirectly its filter-feeding capacity may strip phytoplankton from the water and alter the ecology of the invaded area. Certainly the species deserved the tag of a "pest" species in Port Phillip Bay, although Clapin and Davis (1995), in their study of the Cockburn Sound population, found no evidence that the fanworm was directly threatening any fishery in Western Australia, nor was there any evidence of an obvious impact on native species (Clapin and Evans, 1995).

The aim of this project was to throw some light on the genetics of the species, in particular to confirm genetically that the Australian populations really were *S. spallanzanii*, to determine the genetic relationships among Australian populations, and to see how closely related they were to European populations. There had been no previous genetic studies of this species, neither in Australia nor overseas.

3.   PRINCIPAL AIMS OF PROJECT

1. Confirmation of species identification of the fanworm. The taxonomic confusion surrounding *Sabella spallanzanii*, together with its morphological variability, have raised questions about whether the introduced species is really *S. spallanzanii* or another sabellid. Genetic comparisons of Australian specimens with European specimens of *S. spallanzanii* and with specimens of one or more other species of *Sabella* should resolve this issue.

Should the fanworm prove to be *S. spallanzanii*, then:

2. Assessment of the likelihood of single or multiple introductions, and determination of likely source populations. Genetic uniformity among Australian populations would argue for a single introduction from Europe followed by a spread, genetic heterogeneity would argue for multiple introductions or very limited and differential subsampling from a single introduction. Very close relationships between Australian populations and a particular European population would make that European population a more likely source than less closely-related European populations.

3. The analysis will determine whether the introduction has been accompanied by a loss of genetic variation due to the presumed severe bottleneck in population size (the 'founder' effect). This could prove to be an important piece of information if genetic techniques of control are considered, since it

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could prove simpler to control a genetically monomorphic population than a polymorphic population.

4. METHODS

4.1 COLLECTIONS

In Australia, collections of *Sabella spallanzanii* were made from three States (Western Australia, South Australia, and Victoria). In Europe, Mediterranean collections came from France (Marseille and nearby Carteau) and Spain (Alicante), and an Atlantic collection from France (Roscoff). Further details are provided in Figure 1 and Table 1.

One collection (n = 10) of *S. pavonina* was made, from Roscoff.

After collection, worms were removed from their tubes and the anterior 10 cm wrapped in aluminium foil and frozen. Mediterranean samples were stored at -20°to -35°C for several weeks and Roscoff samples stored in liquid nitrogen for a few days before being airfreighted in dry-ice to Hobart. Australian samples were collected and immediately dispatched to Hobart, either in liquid nitrogen (Victoria) or on dry-ice (South Australia, Western Australia). At Hobart, all samples were maintained at -80°C.

4.2 ELECTROPHORETIC PROCEDURES

Extracts of longitudinal muscle were prepared by grinding small tissue fragments in two drops of homogenizing solution in a 1.5 ml microcentrifuge tube and spinning at 10,000 g for 3 min. The homogenizing solution comprised 100 ml distilled water, 10 mg NADP and 100 microlitres of β-mercaptoethanol. Sample preparation was carried out at 4°C. The supernatant was used for electrophoresis. All electrophoresis runs were carried out using Helena Titan III cellulose acetate plates. Eighteen enzyme systems, representing 23 loci, were examined either with a 200V tris-glycine (Hebert & Beaton 1989) or 150 V tris-citrate (75mM tris, 25mM citric acid, pH 7.0) buffer system (Table 2). Standard staining procedures were followed (Richardson et al. 1986; Hebert and Beaton 1989). When an enzyme was encoded by two loci, the more anodally migrating enzyme was suffixed as 1. Alleles were numbered according to the mobilities of their products relative to that of the most common allele in Victoria, numbered 100.

Several enzymes, including acid phosphatase, adenylate kinase, alcohol dehydrogenase, aldehyde oxidase, aldolase, diaphorase, fumarase, glucose-6-phosphate dehydrogenase, α-glycerophosphate dehydrogenase, hexokinase, octanol dehydrogenase, octopine dehydrogenase, pyruvate kinase, and triose phosphate isomerase either didn't stain or could not be routinely scored and therefore are not considered further.

FIGURE 1. Sampling localities
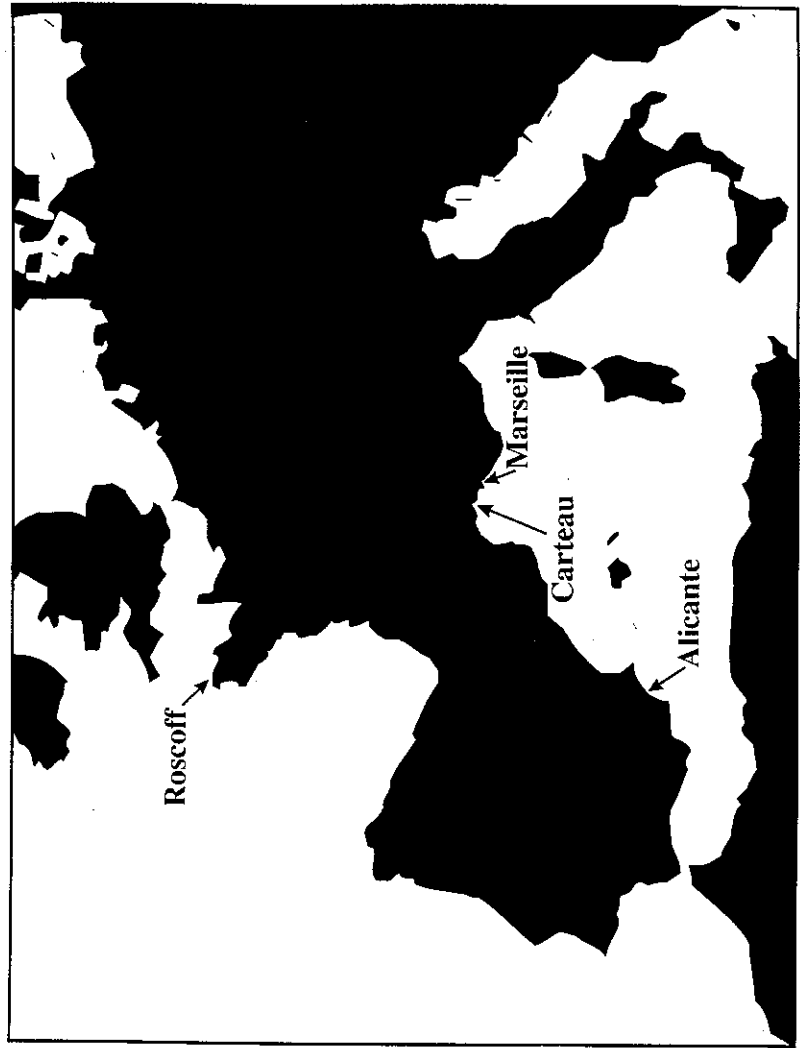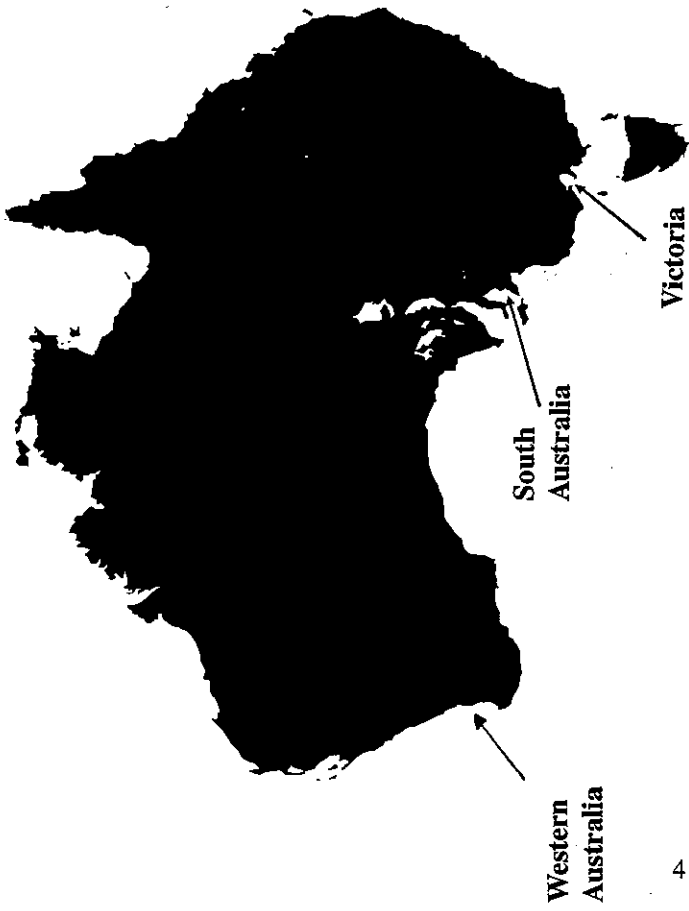
Table 1. *S. spallanzanii* and *S. pavonina*. Sampling sites and dates

Location	Description	Date	Sample size	Long. Lat.
Australia				
Victoria	Point Wilson Pier, near Geelong, Port Phillip Bay	September 1995	108	144°32'E 38°5'S
South Australia	North Haven Boat Harbour, Adelaide	November 1995	100	138°29'E 34°47'S
Western Australia	Southern Flats, Cockburn Sound, near Fremantle	December 1995	100	115°42'E 32°5'S
Europe				
Roscoff	Car ferry port	January 1996	62 (+10 *S.pavonina*)	48°43'N 3°58'W
Carteau	Golfe de Fos, Rhone River estuary	October 1995	48	43°22'N 4°52'E
Marseille	Marseille Harbour	October 1995	50	43°19'N 5°21'E
Alicante	(i) Cabo Roig (Red Cape), Torrevieja (natural site)	December 1995	30	37°54'N 0°43'W
	(ii) Torrevieja Harbour (polluted)	December 1995	30	37°56'N 0°41'W

4.3   STATISTICS

In order to examine whether the observed genotype numbers accorded with those expected in a sexually reproducing population, Hardy-Weinberg tests were carried out. Observed and Hardy-Weinberg expected numbers were compared with chi-square tests. Tests were only considered valid if all cells in a test had an expected value of 1 or greater. This frequently necessitated pooling rarer alleles, producing two allele tests (with one degree of freedom).

Analysing the degree of genetic diversity among collections was carried out in several ways:

1. Contingency chi-square tests were used to determine whether allele frequencies of variable loci were homogeneous over collections. The Monte Carlo randomisation approach of Roff and Bentzen (1989) was used. This eliminates the need to pool rare alleles. One thousand randomisations were carried out for each test. The number of times each of the randomised replicates was greater than or equal to the observed value divided by 1000 was an estimate of the probability of obtaining the observed value by chance.

2. Nei's (1973) gene diversity (G_{ST}) statistics were used to quantify the extent of differentiation among collections. G_{ST} is equal to $(H_T-H_S)/H_T$, where H_T (total genetic diversity) is the average of the Hardy-Weinberg expected heterozygosity across all collections and H_S (mean genetic diversity per area) is the average Hardy-Weinberg expected heterozygosity within collections. Across all loci, G_{ST} was estimated from the mean of the H_T and H_S values. The G_{ST} value represents the proportion of genetic diversity that can be attributed to differences between collections. A bootstrapping procedure (Elliott and Ward, 1992) was used to estimate the magnitude of G_{ST} that could be attributed to sampling error alone. This quantity is termed $G_{ST-null}$ and a mean value of $G_{ST-null}$ was estimated for each locus from 1000 replications. The number of times each of the 1000 estimates of $G_{ST-null}$ was equal to or greater than the observed G_{ST} was determined, and this divided by 1000 gave the probability of obtaining the observed G_{ST} by chance.

3. Relationships between collections were assessed in two ways. The first used Nei's (1978) unbiased genetic distance measure. The resulting genetic distance matrix was converted to a dendrogram of collection relationships by cluster analysis using the UPGMA (unweighted pair-group method with averaging) algorithm. This method assumes a constant rate of evolution. Nei's genetic distance takes a range of 0 (total similarity) to infinity (total dissimilarity), and his unbiased estimates take sample size into account. The second uses Rogers (1972) genetic distance method, and the derived dendrogram is a Wagner tree rooted by the out-group (*S. pavonina*). This method does not assume that all lineages have been evolving at a constant rate. Rogers distance takes a range of 0 to 1, and, being based on allele frequencies alone, does not take collection size into account.

In all analyses involving multiple tests, such as testing for the same effect in each of several loci, the Bonferroni technique was used to adjust significance levels. The predetermined experimentwise significance level, α, of 0.05 was divided by the number of tests within a population to obtain a corrected significance level.

5. RESULTS

5.1 *SABELLA SPALLANZANII* AND *S. PAVONINA*: OVERALL DESCRIPTION OF GENETIC VARIABILITY

Enzymes encoded by a total of 23 loci provided clear and reproducible results. Subunit numbers (Table 2), with one exception, were as listed in Ward et al. (1992). The exception was *Apk*. Ward et al (1992) say "This enzyme is a monomer in arthropods and molluscs, but in annelids and echinoderms it appears to be a dimer". *Sabella* is an annelid, but banding patterns for the *Apk**115/100 heterozygote were clearly five-banded, indicating a tetramer. The much more common heterozygote, *Apk**100/95, gave a broad band of activity, presumably because the five bands were too close together to separate adequately.

The two Alicante collections, one from a polluted site and the other from an unpolluted site, showed differences in allele frequencies (data not shown) at only one of 16 variable loci. This was *Pep-lgg* (P = 0.016), but following Bonferroni correction for multiple tests, the differences were not statistically significant (α altered from 0.05 to 0.05/16 = 0.003). Thus these two neighbouring collections are pooled in all subsequent analyses.

The two collections from the south of France (Carteau and Marseille) were separated by a greater geographic distance than the two Alicante collections, and showed significant differences in gene frequencies at one of the 18 variable loci (*Me-1*, $P<0.001$, α = 0.05/18 = 0.003). These two collections were kept separate in all analyses.

Sabella spallanzanii showed abundant genetic variation (Table 3). Only five loci were monomorphic in all collections (*EstD-1, Ldh, Me-2, Pep-vl-2, Sdh*). The percentage of polymorphic loci ranged from about 45% to 60% (0.95 criterion) or 50% to 80% (0.99 criterion), and the mean Hardy-Weinberg expected heterozygosity per locus ranged from about 0.20 to 0.27 (Table 4).

Genotype distributions within collections of *S. spallanzanii* showed good agreement with Hardy-Weinberg expectations (Table 5). A total of 66 valid tests were performed, and only two showed probabilities of agreement less than 0.05. These were *Pep-vl-1* in South Australia (P = 0.0006), and *Pgi* in Carteau (P = 0.024). Corrections of α levels for 66 tests reduced α to 0.0008, so the *Pgi* result becomes non-significant while the *Pep-vl-1* result (with a heterozygote deficiency: 30 heterozygotes observed, 45.8 expected) remains statistically

Table 2. *S. spallanzanii* and *S. pavonina*. Enzymes, loci, electrophoresis conditions, and likely subunit numbers as assessed from heterozygote banding patterns.

Enzyme	EC number	Locus	Buffer	Run time (mins)	Subunit number
Aspartate aminotransferase	2.6.1.1	*Aat-1, Aat-2*	B	75	2
Aconitase	4.2.1.3	*Acon*	B	60	1
Arginine phosphokinase	2.7.3.3	*Apk*	B	100	4
Esterase D	3.1.1.-	*EstD-1, EstD-2*	A	20	2*
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*G3pdh*	B	75	4
Isocitrate dehydrogenase	1.1.1.42	*Idh*	B	75	2
Lactate dehydrogenase	1.1.1.27	*Ldh*	A	20	invariant
Malate dehydrogenase	1.1.1.37	*Mdh*	B	90	2
Malic enzyme	1.1.1.40	*Me-1,Me-2*	A	30	uncertain
Mannosephosphate isomerase	5.3.1.8	*Mpi*	A	25	1
Peptidase (val-leu)	3.4.11.-	*Pep-vl-1, Pep-vl-2*	B	65	2*
Peptidase (leucine-glycine-glycine)	3.4.11.-	*Pep-lgg*	B	75	1
Peptidase (phenylalanine-proline)	3.4.11.-	*Pep-pp*	A	100	2
6-Phosphogluconate dehydrogenase	1.1.1.44	*6Pgdh*	B	55	2
Glucosephosphate isomerase	5.3.1.9	*Pgi*	A	30	2
Phosphoglucomutase	5.4.2.2	*Pgm-1, Pgm-2*	A	28	1
Sorbitol (iditol) dehydrogenase	1.1.1.14	*Sdh*	A	30	invariant
Xanthine dehydrogenase	1.2.3.2	*Xdh*	A	35	2

*EstD-2 is a dimer, *EstD-1* is invariant. *Pep-vl-1* is a dimer, *Pep-vl-2* is invariant
A=Tris-glycine; B=Tris-citrate

Table 3. *S. spallanzanii* and *S. pavonina*. Allele frequencies, sample sizes (n = number of individuals sampled), and locus heterozygosities (het. = Hardy-Weinberg expected heterozygosity)

Locus	allele	Sabella spallanzanii							Sabella pavonina
		Victoria	South Australia	Western Australia	Roscoff	Carteau	Marseille	Alicante	
Aat-1	110	0	0	0	0.008	0.010	0	0	0
	100	0.949	0.960	0.900	0.992	0.979	1	0.992	0
	95	0	0	0	0	0	0	0	0.050
	93	0	0	0	0	0	0	0	0.900
	90	0.051	0.040	0.100	0	0.010	0	0.008	0.050
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0.097	0.077	0.18	0.016	0.041	0	0.016	0.185
Aat-2	110	0	0	0	0	0	0	0	0.250
	105	0	0	0	0	0.031	0.010	0.042	0.650
	100	1	1	1	1	0.969	0.990	0.958	0.100
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0	0	0	0	0.06	0.02	0.081	0.505
Acon	105	0.153	0.245	0.180	0.097	0.083	0.070	0.075	0
	100	0.792	0.680	0.725	0.476	0.604	0.690	0.800	1
	95	0.056	0.075	0.095	0.427	0.313	0.240	0.125	0
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0.346	0.472	0.433	0.582	0.530	0.461	0.339	0
Apk	115	0	0	0	0	0	0.020	0.025	0
	100	0.741	0.725	0.790	1	0.958	0.940	0.867	0
	95	0.259	0.275	0.210	0	0.042	0.040	0.108	1
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0.384	0.399	0.332	0	0.081	0.114	0.236	0
EstD-1	100	1	1	1	1	1	1	1	1
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0	0	0	0	0	0	0	0
EstD-2	115	0	0	0	0.032	0.149	0.130	0.083	0
	105	0	0	0	0	0	0	0	1
	100	1	1	1	0.968	0.851	0.870	0.917	0
	n	*108*	*100*	*100*	*62*	*47*	*50*	*60*	*10*
	het.	0	0	0	0.062	0.254	0.226	0.152	0
G3pdh	100	0.481	0.420	0.425	0.766	0.833	0.910	0.617	0
	80	0	0	0	0	0.042	0.030	0.150	0
	70	0	0	0	0	0	0	0	0.950
	60	0.051	0.105	0.105	0	0.031	0	0.017	0
	50	0	0	0	0	0	0	0	0.050
	40	0.468	0.475	0.470	0.234	0.094	0.060	0.217	0
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0.547	0.587	0.588	0.359	0.295	0.167	0.549	0.095

Idh	100	0.977	1	0.980	1	0.948	0.960	0.950	1
	95	0.023	0	0.020	0	0.052	0.040	0.050	0
	n	108	100	100	62	48	50	60	10
	het.	0.045	0	0.039	0	0.099	0.077	0.095	0
Ldh	100	1	1	1	1	1	1	1	1
	n	108	100	100	62	48	50	60	2
	het.	0	0	0	0	0	0	0	0
Mdh	115	0.093	0.040	0.040	0	0.010	0.010	0.033	0
	100	0.907	0.960	0.960	1	0.990	0.990	0.967	1
	n	108	100	100	62	48	50	60	10
	het.	0.169	0.077	0.077	0	0.02	0.02	0.064	0
Me-1	105	0.014	0	0	0	0.042	0.170	0.008	0
	100	0.986	1	1	1	0.833	0.810	0.933	0
	95	0	0	0	0	0.125	0.020	0.058	0
	90	0	0	0	0	0	0	0	1
	n	108	100	100	62	48	50	60	10
	het.	0.028	0	0	0	0.289	0.315	0.126	0
Me-2	100	1	1	1	1	1	1	1	1
	n	108	100	100	62	48	50	60	10
	het.	0	0	0	0	0	0	0	0
Mpi	108	0	0	0	0	0	0	0	0.050
	105	0	0	0	0	0.031	0.040	0	0
	103	0	0	0	0	0	0	0	0.950
	100	0.519	0.620	0.480	0.427	0.583	0.540	0.583	0
	95	0.481	0.380	0.520	0.573	0.333	0.410	0.408	0
	90	0	0	0	0	0.052	0.010	0.008	0
	n	108	100	100	62	48	50	60	10
	het.	0.499	0.471	0.499	0.489	0.546	0.539	0.494	0.095
Pep-vl-1	110	0	0	0	0.210	0	0	0.017	0
	100	0.767	0.633	0.710	0.323	0.771	0.796	0.808	0
	90	0.233	0.367	0.290	0.468	0.229	0.204	0.175	0
	75	0	0	0	0	0	0	0	0.250
	70	0	0	0	0	0	0	0	0.750
	n	105	98	100	62	48	49	60	10
	het.	0.357	0.465	0.412	0.633	0.353	0.325	0.316	0.375
Pep-vl-2	130	0	0	0	0	0	0	0	0.200
	115	0	0	0	0	0	0	0	0.800
	100	1	1	1	1	1	1	1	0
	n	108	100	100	62	48	50	60	10
	het.	0	0	0	0	0	0	0	0.320
Pep-lgg	145	0	0	0	0	0	0	0	0.750
	135	0	0	0	0	0	0	0	0.050
	125	0	0	0	0	0	0	0	0.200
	110	0.329	0.405	0.380	0	0.011	0	0	0
	100	0.583	0.535	0.560	0.185	0.383	0.470	0.475	0
	90	0.088	0.060	0.060	0.815	0.585	0.480	0.500	0
	80	0	0	0	0	0.021	0.050	0.025	0
	n	108	100	100	62	47	50	60	10
	het.	0.544	0.546	0.538	0.302	0.511	0.546	0.524	0.395

Pep-pp	115	0.088	0.105	0.120	0.210	0.188	0.245	0.217	0
	110	0.259	0.170	0.265	0.274	0.365	0.298	0.392	0
	100	0.426	0.550	0.355	0.444	0.302	0.372	0.283	0.500
	90	0.227	0.175	0.260	0.065	0.125	0.074	0.108	0.500
	80	0	0	0	0.008	0.021	0.011	0	0
	n	*108*	*100*	*100*	*62*	*48*	*47*	*60*	*8*
	het.	0.692	0.627	0.722	0.679	0.724	0.707	0.708	0.500
6Pgdh	105	0	0	0	0	0.010	0.010	0.017	0.050
	100	0.903	0.910	0.905	0.798	0.792	0.830	0.775	0.400
	98	0	0	0	0	0	0	0	0.500
	95	0.097	0.090	0.095	0.202	0.198	0.150	0.200	0
	90	0	0	0	0	0	0.010	0.008	0.05
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0.175	0.164	0.172	0.322	0.333	0.288	0.359	0.585
Pgm-1	105	0.074	0.070	0.050	0	0	0.010	0.008	0
	100	0.926	0.930	0.950	1	0.979	0.970	0.983	0.050
	90	0	0	0	0	0.021	0.020	0.008	0.850
	85	0	0	0	0	0	0	0	0.100
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0.137	0.13	0.095	0	0.041	0.059	0.034	0.265
Pgm-2	110	0	0	0	0.040	0.277	0.316	0.083	0.750
	100	1	1	1	0.524	0.596	0.602	0.817	0
	90	0	0	0	0.371	0.117	0.061	0.067	0.250
	85	0	0	0	0.065	0.011	0.020	0.033	0
	n	*108*	*100*	*100*	*62*	*47*	*49*	*60*	*10*
	het.	0	0	0	0.582	0.554	0.534	0.320	0.375
Pgi	135	0	0	0	0.121	0.031	0.010	0.033	0
	125	0	0	0	0.097	0.229	0.160	0.217	0
	115	0	0	0	0.016	0.083	0.030	0.042	0
	100	0.954	0.945	0.945	0.226	0.500	0.630	0.467	0
	95	0	0	0	0	0	0	0.025	0.500
	90	0	0	0	0	0.052	0.040	0.050	0
	80	0.046	0.055	0.055	0.540	0.104	0.130	0.158	0.300
	70	0	0	0	0	0	0	0.008	0.200
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0.088	0.104	0.104	0.633	0.676	0.558	0.704	0.62
Sdh	115	0	0	0	0	0	0	0	1
	100	1	1	1	1	1	1	1	0
	n	*108*	*100*	*100*	*62*	*48*	*50*	*60*	*10*
	het.	0	0	0	0	0	0	0	0
Xdh	125	0	0	0	0	0	0	0	1
	110	0	0	0	0.145	0	0.040	0.045	0
	105	0.079	0.060	0.155	0.306	0.188	0.250	0.295	0
	100	0.611	0.600	0.555	0.532	0.438	0.420	0.295	0
	85	0.269	0.305	0.245	0.016	0.250	0.240	0.205	0
	80	0.042	0.035	0.045	0	0.125	0.050	0.161	0
	n	*108*	*100*	*100*	*62*	*48*	*50*	*56*	*10*
	het.	0.546	0.542	0.606	0.602	0.695	0.699	0.756	0

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Table 4. *S. spallanzanii* and *S. pavonina*. Summary of genetic variability in all collections (± standard errors) for 23 loci. Two definitions of polymorphism used, one where the most common allele has a frequency ≤0.95, and one where the most common allele has a frequency ≤0.99

Sample	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic*		Mean HW expected heterozygosity per locus
			0.95	0.99	
S. spallanzanii					
Victoria	107.9 ± 0.1	2.0 ± 0.2	52.17	65.22	0.203 ± 0.048
South Australia	99.9 ± 0.1	1.9 ± 0.2	47.83	56.52	0.204 ± 0.050
Western Australia	100.0 ± 0.0	1.9 ± 0.2	52.17	60.87	0.210 ± 0.051
Roscoff	62.0 ± 0.0	2.0 ± 0.3	43.48	47.83	0.231 ± 0.058
Carteau	47.9 ± 0.1	2.7 ± 0.3	56.52	78.26	0.268 ± 0.055
Marseille	49.8 ± 0.1	2.7 ± 0.3	56.52	73.91	0.248 ± 0.053
Alicante	59.8 ± 0.2	2.8 ± 0.3	60.87	73.91	0.257 ± 0.054
S. pavonina					
Roscoff	9.8 ± 0.2	1.8 ± 0.2	52.17	52.17	0.198 ± 0.049

Table 5. *S. spallanzanii* and *S. pavonina*. Results of tests for Hardy-Weinberg equilibrium within collections.

Population	locus	test valid?	number of alleles observed	for test	chi-square	d.f.	P
S. spallanzanii							
Victoria	Aat-1	no	2	-	-	-	-
	Acon	yes	3	2	0.669	1	0.413
	Apk	yes	2	2	0.007	1	0.935
	G3pdh	yes	3	2	0.176	1	0.675
	Idh	no	2	-	-	-	-
	Mdh	no	2	-	-	-	-
	Me-1	no	2	-	-	-	-
	Mpi	yes	2	2	1.417	1	0.234
	Pep-vl-1	yes	2	2	3.413	1	0.065
	Pep-lgg	yes	3	2	0.062	1	0.804
	Pep-pp	yes	4	3	1.699	3	0.637
	6pgdh	no	2	-	-	-	-
	Pgm-1	no	2	-	-	-	-
	Pgi	no	2	-	-	-	-
	Xdh	yes	4	3	3.346	3	0.341
South	Aat-1	no	2	-	-	-	-
Australia	Acon	yes	3	2	0.271	1	0.603
	Apk	yes	2	2	0.054	1	0.816
	G3pdh	yes	3	3	1.274	3	0.735
	Mdh	no	2	-	-	-	-
	Mpi	yes	2	2	0.083	1	0.773
	Pep-vl-1	yes	2	2	11.787	1	0.001
	Pep-lgg	yes	3	2	0.040	1	0.841
	Pep-pp	yes	4	4	2.961	6	0.814
	6pgdh	no	2	-	-	-	-
	Pgm-1	no	2	-	-	-	-
	Pgi	no	2	-	-	-	-
	Xdh	yes	4	2	0.134	1	0.714
Western	Aat-1	no	2	-	-	-	-
Australia	Acon	yes	3	2	1.852	1	0.604
	Apk	yes	2	2	1.987	1	0.159
	G3pdh	yes	3	3	2.743	3	0.433
	Idh	no	2	-	-	-	-
	Mdh	no	2	-	-	-	-
	Mpi	yes	2	2	0.001	1	0.973
	Pep-vl-1	yes	2	2	3.232	1	0.072
	Pep-lgg	yes	3	2	0.824	1	0.364
	Pep-pp	yes	4	4	2.533	6	0.865
	6pgdh	no	2	-	-	-	-
	Pgm-1	no	2	-	-	-	-
	Pgi	no	2	-	-	-	-
	Xdh	yes	4	3	3.894	3	0.273

Roscoff	*Aat-1*	no	2	-	-	-	-
	Acon	yes	3	2	0.215	1	0.643
	EstD-2	no	2	-	-	-	-
	G3pdh	yes	2	2	3.709	1	0.054
	Mpi	yes	2	2	0.011	1	0.916
	Pep-vl-1	yes	3	3	0.811	3	0.847
	Pep-lgg	yes	2	2	0.002	1	0.961
	Pep-pp	yes	5	3	2.417	3	0.490
	6Pgdh	yes	2	2	1.545	1	0.214
	Pgm-2	yes	4	2	2.197	1	0.138
	Pgi	yes	5	3	5.863	3	0.118
	Xdh	yes	4	3	5.663	3	0.129
Carteau	*Aat-1*	no	2	-	-	-	-
	Aat-2	no	2	-	-	-	-
	Acon	yes	3	2	2.468	1	0.116
	Apk	no	2	-	-	-	-
	EstD-2	no	2	-	-	-	-
	G3pdh	yes	4	2	0.077	1	0.781
	Idh	no	2	-	-	-	-
	Mdh	no	2	-	-	-	-
	Me-1	yes	3	2	0.077	1	0.781
	Mpi	yes	4	2	0.016	1	0.900
	Pep-vl-1	yes	2	2	0.220	1	0.639
	Pep-lgg	yes	4	2	0.339	1	0.561
	Pep-pp	yes	5	2	0.212	1	0.646
	6Pgdh	yes	3	2	3.133	1	0.077
	Pgm-1	no	2	-	-	-	-
	Pgm-2	yes	4	2	0.893	1	0.345
	Pgi	yes	6	3	9.470	3	0.024
	Xdh	yes	4	3	0.841	3	0.840
Marseille	*Aat-2*	no	2	-	-	-	-
	Acon	yes	3	2	2.335	1	0.126
	Apk	no	3	-	-	-	-
	EstD-2	no	2	-	-	-	-
	G3pdh	no	3	-	-	-	-
	Idh	no	2	-	-	-	-
	Mdh	no	2	-	-	-	-
	Me-1	yes	3	2	1.404	1	0.236
	Mpi	yes	4	2	0.774	1	0.379
	Pep-vl-1	yes	2	2	0.001	1	0.971
	Pep-lgg	yes	3	2	0.624	1	0.430
	Pep-pp	yes	5	3	1.755	3	0.625
	6Pgdh	yes	4	2	0.145	1	0.703
	Pgm-1	no	3	-	-	-	-
	Pgm-2	yes	4	2	2.481	1	0.115
	Pgi	yes	6	3	5.688	3	0.128
	Xdh	yes	5	3	2.608	3	0.456

Alicante	*Aat-1*	no	2	-	-	-	-
	Aat-2	no	2	-	-	-	-
	Acon	yes	3	2	0.308	1	0.579
	Apk	yes	3	2	0.000	1	0.992
	EstD-2	no	2	-	-	-	-
	G3pdh	yes	4	3	2.013	3	0.570
	Idh	no	2	-	-	-	-
	Mdh	no	2	-	-	-	-
	Me-1	no	3	-	-	-	-
	Mpi	yes	3	2	0.822	1	0.365
	Pep-vl-1	yes	3	2	0.011	1	0.915
	Pep-lgg	yes	3	2	2.606	1	0.106
	Pep-pp	yes	4	3	0.072	3	0.995
	6Pgdh	yes	4	2	0.502	1	0.479
	Pgm-1	no	3	-	-	-	-
	Pgm-2	yes	4	2	0.674	1	0.412
	Pgi	yes	8	4	4.010	6	0.675
	Xdh	yes	4	4	5.393	6	0.495
Sabella	*Aat-1*	no	2	-	-	-	-
pavonina	*Aat-2*	yes	3	2	0.022	1	0.882
	G3pdh	no	2	-	-	-	-
	Mpi	no	2	-	-	-	-
	Pep-vl-1	no	2	-	-	-	-
	Pep-vl-2	no	2	-	-	-	-
	Pep-lgg	no	3	-	-	-	-
	Pep-pp	yes	2	2	0.356	1	0.551
	6pgdh	yes	4	2	11.111	1	0.001
	Pgm-1	no	3	-	-	-	-
	Pgm-2	no	2	-	-	-	-
	Pgi	yes	3	2	0.218	1	0.641

significant. However, whether this deviation represents a real biological phenomenon or simply a chance sampling artefact is uncertain: repeat sampling from that population would be necessary to discriminate between these possibilities. Only ten *S. pavonina* were sampled, and these were not tested for conformance to Hardy-Weinberg expectations.

The three Australian collections were very similar to one another in levels of variation. Among the European collections, the Mediterranean collections were very similar to one another in levels of variation, and were more variable than the Roscoff collections (Table 4).

The Australian collections were clearly less variable, over all loci, than the European collections (Table 4). Mean numbers of alleles per locus were around 2.0 in the Australian collections, compared with between 2.0 and 2.8 in the overseas collections. The average sample size for the overseas collections was slightly greater than half that of the Australian collections: had the overseas sample sizes been more similar to the Australian sample sizes, it is possible that this difference would have been accentuated. The mean percentage of loci polymorphic in the Australian collections ranged from 47.8 to 52.2 (0.95 criterion) and 56.5 to 65.2 (0.99 criterion), values again generally less than in the overseas collections, 43.5 to 60.9 (0.95) and 47.8 to 78.3 (0.99).

The best single parameter for comparing variation is mean Hardy-Weinberg expected heterozygosity per locus (H), as it is far less sample-size dependent than either mean numbers of alleles per locus or mean percentage of loci polymorphic. H for the Australian collections ranges from 0.203 to 0.210, compared with 0.231 to 0.268 for the overseas collections. The mean H of the Australian collections was 0.206, about 18% less than the mean of the four European collections, 0.251.

This loss of variation is by no means distributed uniformly over all loci. Indeed some loci (*Aat-1*, *Apk*, *G3pdh*, *Mdh*, *Pgm-1*) show more variation in the Australian collections. However, this is more than offset by those loci that are more variable in Europe (*EstD-2*, *Me-1*, *6Pgdh*, *Pgm-2*, *Pgi*, *Xdh*). Particularly striking losses of variation in Australia are seen for *Pgm-2* and *Pgi*. *Pgm-2* is monomorphic in Australian collections but highly polymorphic in all European collections tested (heterozygosities ranging from 0.320 to 0.582). *Pgi* shows little variation in Australian collections (heterozygosities ranging from 0.088 to 0.104), but is strikingly variable in European collections (heterozygosities ranging from 0.558 to 0.704).

Among the European collections, the Mediterranean collections showed more variation than the Atlantic collection (Table 4). This is true for all measures of variation: mean numbers of alleles (2.7-2.8 versus 2.0), percentage of loci polymorphic (0.95 criterion, 57-61% vs. 43%; 0.99 criterion, 74-78% vs. 48%), and expected heterozygosity (0.25-0.27 vs. 0.23). Table 6 compares the four

European collections pair-wise, and enumerates the numbers of alleles found in one collection that are not found in another collection. It shows, for example, that the Roscoff collection has 2 alleles not found in Carteau, 2 alleles not found in Marseille, and 2 alleles not found in Alicante. On the other hand, Carteau, Marseille and Alicante have, respectively, 17, 17 and 20 alleles not found at Roscoff.

Table 6. *S. spallanzanii.* Pairwise collection comparisons for unique alleles. Numbers are numbers of alleles present in the 'column' population that are not present in the 'row population.

	Number of unique alleles			
	Roscoff	Carteau	Marseille	Alicante
Roscoff	***********	17	17	20
Carteau	2	***********	4	6
Marseille	2	4	***********	5
Alicante	2	4	2	***********

S. pavonina showed slightly less variability than European *S. spallanzanii,* in terms of average numbers of alleles per locus and expected heterozygosity. However, while the same 23 loci were examined in *S. pavonina,* only about 10 individuals were scored. Thus it is not unexpected that fewer alleles per locus were detected in *S. pavonina*: with a sample size closer to the 50 or so of each of the European *S. spallanzanii,* it is likely that more alleles would have been found.

5.2 *SABELLA SPALLANZANII.* GENETIC RELATIONSHIPS AMONG AUSTRALIAN POPULATIONS.

Fifteen loci were variable in the Australian collections. Gene frequencies were compared among the three collections using the Roff and Bentzen (1989) Monte Carlo chi-square technique with 1000 replicates. The estimated probabilities of no significant differentiation, in descending order, were: 0.985, *6Pgdh*; 0.915, *Pgi*; 0.641, *Pgm-1*; 0.449, *Pep-lgg*; 0.286, *Apk*; 0.203, *G3pdh*; 0.151, *Idh*; 0.104, *Me-1*; 0.063, *Acon*; 0.042, *Xdh*; 0.032, *Mdh*; 0.029, *Aat-1*; 0.012, *Mpi*; 0.007, *Pep-vl-1*; 0.006, *Pep-pp.* Thus six of the 15 loci showed probabilities less than 0.05. However, after carrying out Bonferroni adjustments of α levels for multiple tests (α changes from 0.05 to 0.05/15 = 0.003), none is statistically significant. This

conclusion still holds after carrying out the less conservative sequential Bonferroni test (Hochberg, 1988).

Clearly the three Australian collections, all with a sample size of around 100, are very similar to one another. Indeed, the small differences in allele frequencies, after making Bonferroni corrections, were not statistically significant for any locus.

5.3   *SABELLA SPALLANZANII.* GENETIC RELATIONSHIPS AMONG EUROPEAN POPULATIONS

There were 18 variable loci in the European collections. Eight of these (*Aat-1, Aat-2, EstD-2, Idh, Mdh, 6Pgdh, Pep-pp, Pgm-1*) showed no significant differentiation following use of the Monte-Carlo chi-square procedure (probabilities respectively 0.843, 0.546, 0.528, 0.256, 0.236, 0.078, 0.057, 0.012, with $\alpha = 0.05/18 = 0.003$), and ten (*Acon, Apk, G3pdh, Me-1, Mpi, Pep-lgg, Pep-vl, Pgi, Pgm-2, Xdh*) showed significant differentiation (all with $P<0.001$). So clearly there is significant differentiation among the European collections.

For each of the ten differentiated loci the four collections were compared pairwise, to determine the source(s) of the differentiation (Table 7).

Each comparison was of allele frequencies at 10 loci, and so the α level for within pairwise collection comparisons was reduced from 0.05 to $0.05/10 = 0.005$. The Carteau and Marseille collections, which were only about 40 k apart (Zibrowius, pers. comm.), were significantly different for only one out of the 10 loci, *Me-1*. The Carteau and Marseille collections were significantly differentiated from the remaining Mediterranean collection, Alicante, by three of the ten loci. The Atlantic Roscoff collection, on the other hand, was well differentiated from each of the three Mediterranean collections by between eight and ten loci. Clearly, the Mediterranean collections are genetically more similar to one another than they are to the Atlantic collection.

5.4   *SABELLA SPALLANZANII.* GENETIC RELATIONSHIPS AMONG ALL POPULATIONS

There was extensive spatial differentiation in allele frequencies among collections, with 17 of the 18 variable loci showing significant allele frequency heterogeneity following chi-square analysis of allele frequencies (Table 8). The only variable locus not to show such heterogeneity (following Bonferroni correction to α values, giving $\alpha = 0.05/18 = 0.0028$) was *Idh*.

The extent of the heterogeneity was quantified for each locus by a G_{ST} analysis (Table 8). In this analysis, the observed extent of differentiation among populations (G_{ST}) is compared with sampling error, i.e. the extent of G_{ST} expected in those sample sizes from a panmictic population ($G_{ST}.null$).

Table 7. *Sabella spallanzanii.* Summary of results comparing the significantly differentiated loci among European collections in pairwise comparisons. n.s. indicates $P>0.05$

	Locus	Roscoff	Carteau	Marseille
Carteau	*Acon*	n.s.		
	Apk	0.027		
	G3pdh	<0.001		
	Me-1	<0.001		
	Mpi	<0.001		
	Pep-vl-1	<0.001		
	Pep-lgg	<0.001		
	Pgm-2	<0.001		
	Pgi	<0.001		
	Xdh	<0.001		
Marseille	*Acon*	0.003	n.s.	
	Apk	0.003	n.s.	
	G3pdh	<0.001	n.s.	
	Me-1	<0.001	<0.001	
	Mpi	0.004	n.s.	
	Pep-vl-1	<0.001	n.s.	
	Pep-lgg	<0.001	n.s.	
	Pgm-2	<0.001	n.s.	
	Pgi	<0.001	n.s.	
	Xdh	<0.001	n.s.	
Alicante	*Acon*	<0.001	<0.001	n.s.
	Apk	<0.001	0.027	n.s.
	G3pdh	<0.001	<0.001	<0.001
	Me-1	0.004	n.s.	<0.001
	Mpi	0.009	0.031	n.s.
	Pep-vl-1	<0.001	n.s.	n.s.
	Pep-lgg	<0.001	n.s	n.s.
	Pgm-2	<0.001	<0.001	<0.001
	Pgi	<0.001	n.s.	n.s.
	Xdh	<0.001	0.034	n.s.

Table 8. *S. spallanzanii.* Chi-square and G_{ST} analyses of the extent of differentiation among collections. H_T is the total heterozygosity across all populations, H_S is the average heterozygosity per collection, G_{ST} is the proportion of the variation attributable to collection differences, $G_{ST} \cdot null$ is the expected value of G_{ST} that can be attributed to sampling error alone.

Locus	H_T	H_S	Chi-square	P	G_{ST}	mean $G_{ST} \cdot null$	P
Aat-1	0.066	0.063	42.661	<0.001	0.033	0.006	<0.001
Aat-2	0.023	0.023	27.855	<0.001	0.022	0.006	0.002
Acon	0.484	0.452	146.614	<0.001	0.065	0.007	<0.001
Apk	0.242	0.221	102.234	<0.001	0.089	0.006	<0.001
EstD-1	0	0	-	-	-	-	-
EstD-2	0.106	0.099	84.057	<0.001	0.067	0.007	<0.001
G3pd	0.509	0.442	272.899	<0.001	0.133	0.006	<0.001
Idh	0.052	0.051	16.690	0.016	0.016	0.006	0.022
Ldh	0	0	-	-	-	-	-
Mdh	0.063	0.061	25.534	0.002	0.026	0.006	0.002
Me-1	0.119	0.108	188.268	<0.001	0.093	0.006	<0.001
Me-2	0	0	-	-	-	-	-
Mpi	0.516	0.506	86.542	<0.001	0.020	0.006	0.002
Pep-vl-1	0.448	0.409	238.027	<0.001	0.089	0.006	<0.001
Pep-vl-2	0	0	-	-	-	-	-
Pep-lgg	0.630	0.502	502.686	<0.001	0.203	0.006	<0.001
Pep-pp	0.714	0.694	102.146	<0.001	0.028	0.006	<0.001
6Pgd	0.265	0.259	40.213	0.001	0.021	0.006	0.001
Pgm-1	0.072	0.071	40.252	<0.001	0.024	0.006	<0.001
Pgm-2	0.355	0.284	486.091	<0.001	0.200	0.006	<0.001
Pgi	0.520	0.410	590.180	<0.001	0.212	0.006	<0.001
Sdh	0	0	-	-	-	-	-
Xdh	0.668	0.635	234.446	<0.001	0.049	0.006	<0.001

Of the 18 variable loci, only *Idh* (as in the chi-square analysis) failed to show more inter-collection differentiation than expected by sampling error alone ($P = 0.024 > \alpha = 0.05/18 = 0.003$). The remaining 17 loci all showed significant differentiation, with between about 1% (*Aat-2, Mpi, 6Pgdh*) and 20% (*Pep-lgg, Pgm-2, Pgi*) of the genetic variation arising from inter-collection differences (these figures estimated from G_{ST} - G_{ST} *null*).

Matrices of genetic distance between all pairwise collection comparisons are given in Table 9 (Nei's 1978 and Rogers 1972 measures). A UPGMA tree derived from Nei's distances is given in Fig. 2 and a Wagner tree from Rogers' distances in Fig. 3.

The UPGMA-derived dendrogram of Nei's (1978) genetic distances (Fig. 2) shows:

1. That the Australian collections are, as expected from the earlier analyses (section 5.3), very closely related to one another.

2. That the Mediterranean collections are closely related to one another.

3. That the Atlantic collection is more closely related to the Mediterranean collections than are the Australian collections.

The UPGMA tree separates the Australian collections from the European collections, because the Atlantic and Mediterranean collections are more closely related to one another than either is to the Australian collections. However, the Australian collections are more closely related to the Mediterranean collections (mean genetic distance of 0.040, $n = 9$, range 0.029–0.050, see Table 9) than to the Atlantic collection (mean genetic distance 0.084, $n = 3$, range 0.082–0.086). In fact, the Australian collections are more closely related to the Alicante collection (mean genetic distance 0.032, $n = 3$, range 0.029–0.037) than to Carteau or Marseille (mean genetic distance 0.044, $n = 6$, range 0.038–0.050).

The Wagner tree of Rogers (1972) distances with *S. pavonina* as an outgroup (Fig. 3) shows:

1. That the Australian collections are very closely related to one another and a little separate from other *S. spallanzanii* collections.

2. That the Mediterranean collections are closely related to one another.

3. That the Australian collections are more closely related to the Alicante collection than to any other collections.

The major difference between the UPGMA tree and the Wagner tree concerns the placement of the Australian collections. In the UPGMA tree they cluster away from the European collections, in the Wagner tree they cluster with the Alicante collection. In fact, the close relationship between the Alicante and Australian populations has already been noted. A basic difference between UPGMA and the Wagner trees is that the former assumes a constant rate of

FIGURE 2.
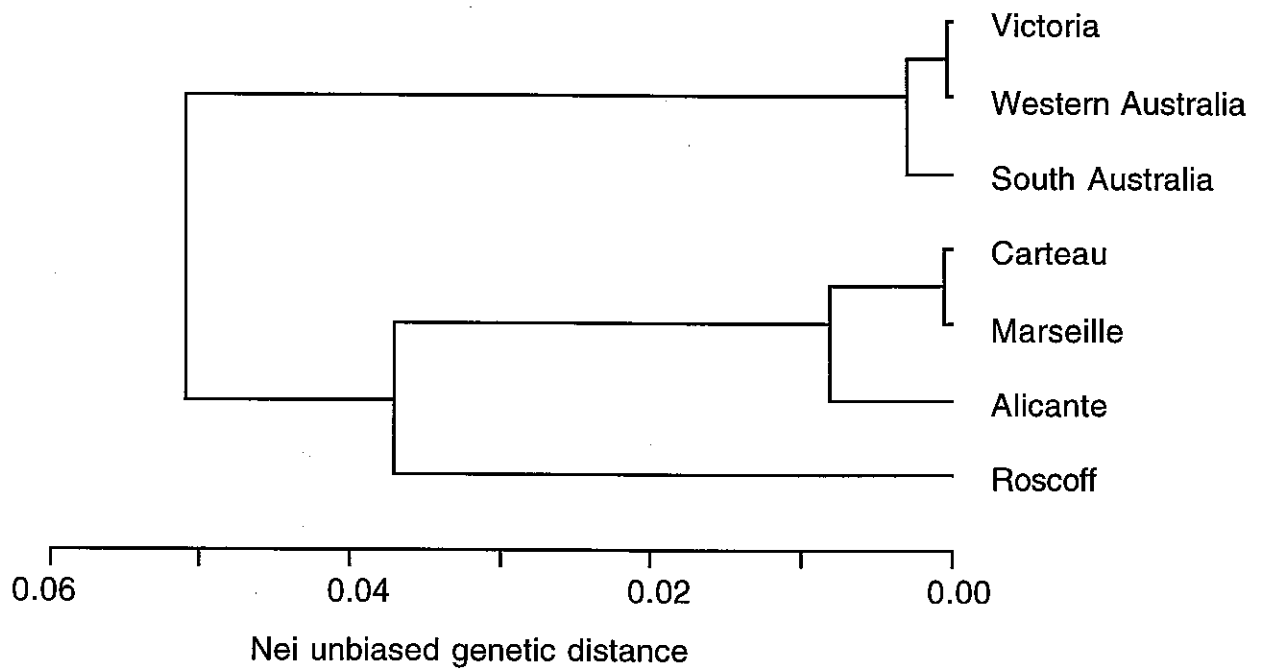
Genetic relationships among *Sabella spallanzanii* populations
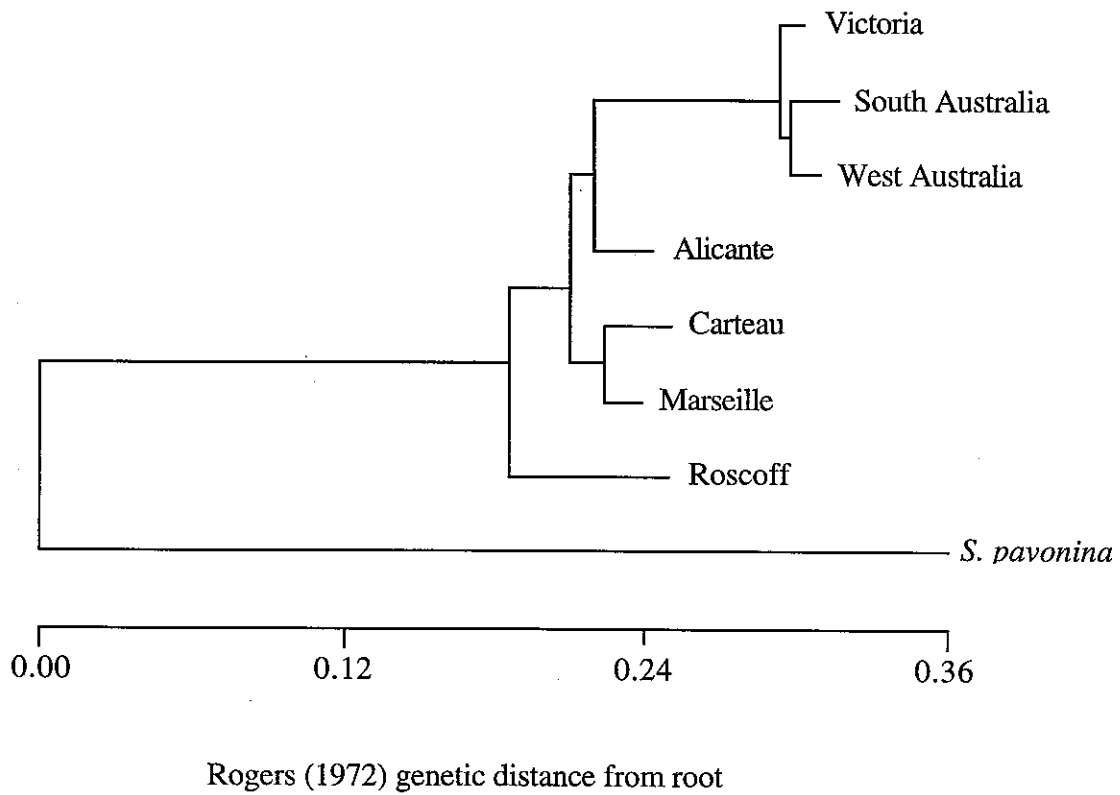
FIGURE 3.

Figure 3. Genetic relationships among *Sabella spallanzanii* populations, using *S. pavonina* as an outgroup.

evolution whereas the latter does not. It may well be that in the case of an introduced species, which has gone through a bottleneck leading to the loss of some genetic variation and therefore accelerated change in gene frequencies, the Wagner tree better represents population relationships by allowing for an enhanced evolutionary rate of the introduced populations.

The greater resemblance of the Australian collections to the Mediterranean collections can also be seen by looking at individual loci. The three most differentiated loci (*Pep-lgg*, *Pgm-2*, and *Pgi*, all with G_{ST} values of around 0.20, see Table 8) may be considered as examples. Allele frequencies for these three loci for the pooled Australian collections, pooled Mediterranean collections, and the Atlantic collection, were calculated (Table 10).

With respect to *Pep-lgg*, the dominant European allele, *Pep-lgg*90*, has a frequency of about 0.82 in the Atlantic, 0.52 in the Mediterranean, but only 0.07 in the Australian collections. The common Australian allele, *Pep-lgg*90*, with a mean frequency of 0.56, has a frequency of around 0.45 in the Mediterranean collections but only 0.19 in the Atlantic collection. The next most abundant Australian allele, *Pep-lgg*110*, with a frequency of about 0.37, is rare in the Mediterranean (frequency less than 0.01) and was not recorded in the Atlantic collection.

With respect to *Pgm-2*, the Australian collections were monomorphic for *Pgm-2*100*, which, while the most common allele in all overseas collections, was more abundant in the Mediterranean collections (frequency 0.68) than in the Atlantic collection (0.52).

With respect to *Pgi*, the Australian collections were almost fixed for an allele (*Pgi*100*) that was the most common in the Mediterranean (frequency 0.53) but was only the second most common allele in the Atlantic collection (with a frequency of 0.23).

5.6 *SABELLA SPALLANZANII* AND *S. PAVONINA*: GENETIC RELATIONSHIPS

S. spallanzanii and *S. pavonina* are genetically very distinct. While only 10 specimens of *S. pavonina* were examined, compared with more than 500 *S. spallanzanii*, fixed differences were observed at 9 loci (*EstD-2*, *G3pdh*, *Me-1*, *Mpi*, *Pep-vl-1*, *Pep-vl-2*, *Pep-lgg*, *Sdh*, *Xdh*) and nearly fixed differences at 3 further loci (*Aat-1*, *Aat-2*, *Pgm-1*). Note that this genetic distinctiveness is not caused by allopatric separation of populations: the *S. pavonina* collection was sampled from Roscoff where it was sympatric with the sampled *S. spallanzanii* population.

Inspection of the genetic distance matrix (Table 9) shows that the mean Nei genetic distance between *S. spallanzanii* and *S. pavonina* is 0.996 ($n = 7$, range 0.987–1.025), while the mean genetic distance among the different
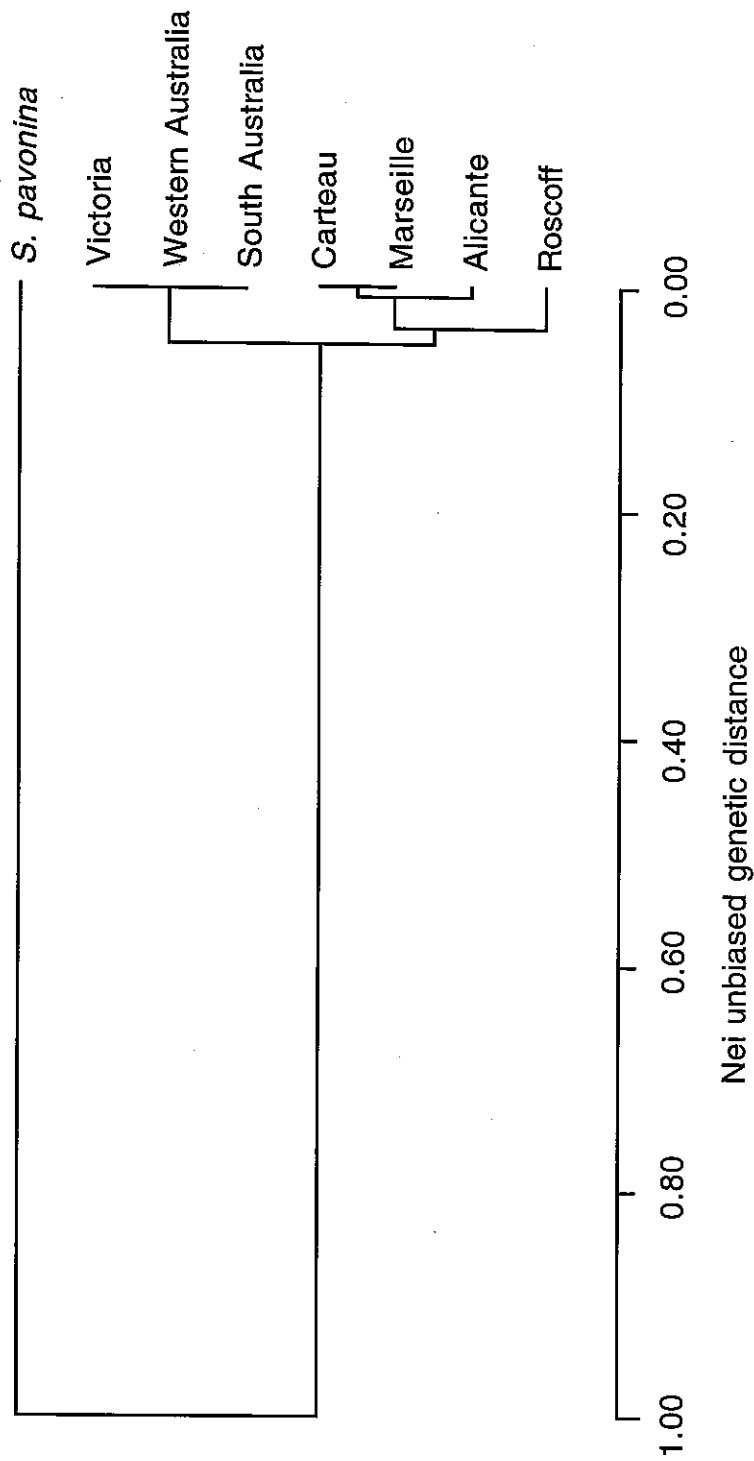
Table 9. *Sabella spallanzanii.* and *S. pavonina.* Matrices of genetic distance (23 loci) among collections. Above diagonal, Nei (1978) unbiased genetic distance, below diagonal, Rogers (1972) genetic distance.

	Victoria	South Australia	Western Australia	Roscoff	Carteau	Marseille	Alicante	*S. pavonina*
			Sabella spallanzanii					
Victoria	-	0.002	0.000	0.086	0.044	0.038	0.029	0.997
South Australia	0.032	-	0.003	0.085	0.050	0.045	0.037	0.993
Western Australia	0.025	0.032	-	0.082	0.045	0.040	0.030	1.003
Roscoff	0.167	0.165	0.158	-	0.032	0.038	0.040	1.025
Carteau	0.133	0.141	0.132	0.109	-	0.001	0.007	0.987
Marseille	0.122	0.133	0.122	0.116	0.040	-	0.009	0.987
Alicante	0.106	0.120	0.107	0.121	0.058	0.062	-	0.981
S. pavonina	0.612	0.611	0.612	0.612	0.601	0.601	0.599	-

Table 10. *Sabella spallanzanii.* Allele frequencies for the three most differentiated loci in pooled Australian collections, pooled Mediterranean collections, and the Atlantic collection.

Locus	Allele	Australia	Mediterranean	Atlantic
Pep-Igg	110	0.370	0.003	0
	100	0.560	0.446	0.185
	90	0.070	0.519	0.815
	80	0	0.032	0
	N	308	157	62
Pgm-2	110	0	0.215	0.040
	100	1.0	0.682	0.524
	90	0	0.080	0.371
	85	0	0.022	0.065
	N	308	156	62
Pgi	135	0	0.023	0.121
	125	0	0.203	0.097
	115	0	0.051	0.016
	100	0.948	0.529	0.226
	95	0	0.009	0
	90	0	0.047	0
	80	0.052	0.133	0.540
	70	0	0.003	0
	N	308	158	62

FIGURE 4.

Genetic relationships between *Sabella spallanzanii* and *S. pavonina*

S. spallanzanii collections was only 0.035 (*n*= 21, range 0.000–0.086). The mean genetic distance between Australian and European collections of *S. spallanzanii* was 0.051 (*n* = 12, range 0.029–0.086). These relationships are confirmed by the UPGMA-derived genetic distance dendrogram (Fig. 4). *S. pavonina* is also well separated from the *S. spallanzanii* collections in the Wagner tree of Rogers genetic distance (Fig. 3). The similarity of the *S. spallanzanii* collections to one another, plus the distinctiveness of *S. pavonina*, indicates very strongly that the Australian collections are indeed *S. spallanzanii* and not another *Sabella* species.

6. DISCUSSION

The first point to be made is that the fanworms examined from Western Australia, South Australia, and Victoria are indeed *Sabella spallanzanii*. Dr Phyllis Knight-Jones, a sabellid expert from the University of Wales, Swansea, came to the same conclusion following a morphological examination of specimens from Port Phillip Bay (quoted in Clapin and Evans, 1995). The morphology of specimens from Cockburn Sound (Western Australia) also indicated that these were *S. spallanzanii* (Clapin and Evans, 1995). Genetically, the samples from Port Phillip Bay, Cockburn Sound, and Adelaide are virtually identical, showing that all are of the same species. Furthermore, they are genetically very closely related to *S. spallanzanii* from Europe, with a mean Nei genetic distance between the two groups of animals of only 0.051. On the other hand, the Nei genetic distance between these populations of *S. spallanzanii* and another sabellid, *S. pavonina*, was a much higher 0.996. There can be no doubt that the Australian specimens are indeed *S. spallanzanii*, and that they derive from one or more European introductions.

Sabella spallanzanii is genetically a very variable species. The average heterozygosity per locus, over the 23 examined loci, for the Mediterranean collections was about 0.25. This is about twice as high as the average heterozygosity per locus for 370 species of marine and terrestrial invertebrates, recorded by Ward *et al.* (1992) as 0.122, although perhaps 10% of invertebrates are more variable still than *S. spallanzanii*.

Among the European collections, the Atlantic Roscoff collection was less variable than the Mediterranean collections, with a mean heterozygosity of around 0.23, a reduction in the average numbers of alleles per locus from around 2.7 to 2.0, and a reduction in the percentage of polymorphic loci (0.99 criterion) from around 75% to 50%. A total of 21 alleles were found in the Mediterranean collections which were not found in the Roscoff collection, but no alleles were found at Roscoff which were not found in the Mediterranean. This asymmetric pattern cannot simply be attributed to more animals having been sampled from the Mediterranean, since even individual Mediterranean

collection, with sample sizes smaller than the Roscoff collection, showed Mediterranean-specific alleles (Carteau, 17; Marseille, 17; Alicante, 20).

While these alleles were all quite rare, generally having frequencies less than 0.10 and therefore contributing relatively little to heterozygosity, their presence in the Mediterranean and absence from Roscoff suggests either that the species was originally native to the Mediterranean and lost rare alleles in the process of spreading to the Atlantic seaboard, or that Mediterranean populations have maintained higher population sizes and therefore more variation than the Atlantic populations. The latter was suggested by Bembo *et al.* (1996) to account for their similar finding of increased allozyme heterozygosity in Mediterranean anchovies (*Engraulis encrasicolus*) compared with an Atlantic sample. Quesada *et al.* (1995a) suggested that for *Mytilus galloprovincialis*, Pleistocene refugia in the Mediterranean maintained higher population sizes than Atlantic populations during glacial maxima, therebye explaining the present-day higher mitochondrial DNA diversity of the former populations. However, despite some allozyme gene frequency differences (Quesada *et al.*, 1995b), there were no differences in overall allozyme heterozygosity between *M. galloprovincialis* populations from the Mediterranean and the Atlantic (Quesada, pers. comm.). Clearly, it would be useful to examine further Mediterranean and, especially, Atlantic populations of *S. spallanzanii* to test these ideas further.

The Australian collections were somewhat less variable than the European collections. While mean numbers of alleles per locus were similar to those of the Roscoff collection, at around 2.0 (compared with about 2.7 for the Mediterranean), sample sizes were substantially higher, meaning that rare alleles were more likely to be detected and counted in the Australian than European collections. Number of alleles and percentage polymorphism are more sample-size dependent than average heterozygosity per locus, and it is this latter parameter that is the best single parameter for estimating levels of genetic variation. The Australian collections had an average heterozygosity about 18% less than the average of the four European collections, and about 20% less than that of the three Mediterranean collections. Two loci in particular, *Pgm-2* and *Pgi*, show striking losses in variation. *Pgm-2* is monomorphic in Australian collections but highly polymorphic in all European collections examined. *Pgi* shows little variation in Australian collections but is highly variable in European collections. It is clear that the introduction from Europe into Australia had been accompanied by the loss of some genetic variation.

Introductions do not inevitably lead to the loss of large amounts of variation. Table 11 summarises some observations made on the genetic effects of the introduction of the Pacific seastar, *Asterias amurensis*, to Tasmanian waters (Ward and Andrew, 1995), and more generally on seven species of introduced molluscs, a group for which most such data exists. All these introductions were accidental, rather than the deliberate transfer of, for example, commercial

Table 11. Losses of genetic variation consequent upon accidental introductions of *Sabella spallanzanii, Asterias amurensis* and seven species of molluscs. Superscripts to average heterozygosity values indicate number of populations examined. Areas in parentheses indicate endemic vs. introduced populations)

Species	number of loci	Average heterozygosity endemic	introd.	% change	Reference
Sabella spallanzanii (Europe vs. Australia)	23	0.251[4]	0.206[3]	-17.9	present study
Asterias amurensis (Japan/Russia vs. Tasmania)	22	0.192[7]	0.123[3]	-35.9	Ward and Andrew, 1995
Marine molluscs					
Littorina saxatilis (N. Atlantic vs. South Africa)	16	0.181[13]	0.052[2]	-70.7	Knight *et al.*, 1987
Littorina saxatilis (N. Atlantic vs. Venice)	16	0.181[13]	0.131[1]	-27.6	ditto
Crepidula onyx (California vs. Hong Kong)	23	0.167[3]	0.141[1]	-15.6	Woodruff *et al.*, 1986
Crepidula fornicata (E. US vs. UK)	24	0.045[9]	0.030[1]	-33.3	Hoagland, 1985
Mytilus galloprovincialis (Spain vs. South Africa)	23	0.24[1]	0.22[1]	-8.3	Grant and Cherry, 1985
Macoma balthica (NW. Atlantic vs. California)	11	0.394[1]	0.417[1]	+5.8	Meehan *et al.*, 1989
Freshwater mollusc					
Dreissena polymorpha (Europe vs. Great Lakes)	11 16	0.451[3] 0.327[7]	0.465[7] 0.370[18]	+3.1 +13.1	Boileau and Hebert, 1993 Marsden *et al.*, 1995
Land mollusc					
Theba pisana (France vs. Australia)	25	0.176[2*]	0.086[2]	-51.1	Johnson, 1988

* excludes 4 endemic populations judged not to be likely source populations.

species of oysters. Three introductions, *Mytilus galloprovincialis, Macoma balthica* and *Dreissena polymorpha*, were not accompanied by the loss of detectable levels of genetic diversity, while two introductions showed striking losses. These are the introduction to South Africa of the intertidal snail *Littorina saxatilis* (Knight *et al.*, 1987), and the introduction to Australia of the land snail *Theba pisana* (Johnson, 1988). Interestingly, these are the only two species of all those listed in Table 10 that do not have planktonic larvae; both are ovoviviparous.

Thus, while it may be that the variation in the amount of genetic loss reflects variation in the number of founders, it may also reflect variation in reproductive mode. Species with planktonic larvae will produce many more offspring than ovoviviparous species, and thus have the potential to increase population size very rapidly. Nei *et al.* (1975) and Chakraborty and Nei (1977) have shown that, following a bottleneck, the reduction in heterozygosity depends not only on the size of the bottleneck but also on the rate of recovery from the bottleneck. Hence, bottleneck effects in species with high fecundity and potentially high rates of population increase may be expected to be less marked than in ovoviviparous species. Differences in the amount of variation lost among species with similar life histories may reflect differences in the original founding population sizes or perhaps the occurrence of multiple versus single introductions.

In fact, the actual effect on average heterozygosity of a founding event *per se* may be rather small. The expected proportion of the original heterozygosity remaining after a bottleneck of size N for one generation is $1-1/(2N)$, so the progeny of one male and one female will alone retain 75% of the original heterozygosity. However, while the initial loss of heterozygosity may be limited, heterozygosity will continue to be lost each generation at the rate of about $1/2N$: heterozygosity in small populations will be lost far faster than in large populations.

The loss of variation in introduced populations of *Sabella spallanzanii* can be attributed both to a founder effect (with small numbers of animals forming the Australian bridgehead) and to likely small numbers of animals in the first few Australian generations.

The transport mechanism that brought *S. spallanzanii* to Australia from Europe is unclear. Certainly the species has been observed attached to ships' hulls (Clapin and Evans, 1995), and it is probable that they can remain attached at least to slow-moving vessels. It has also been noted that specimens can survive short periods of high temperature (12 hours at 30°C) increasing their chances of surviving transport through tropical waters, although stress observed at 27°C makes it unlikely that they will ever colonise warm tropical waters (Clapin, 1996). It is also possible that they can be transported as larvae in ballast water, although their lecithotropic (non-feeding) nature (Giangrande and Petraroli,

1994; Rouse and Fitzhugh, 1994) is likely to mean that their larval stages are relatively short-lived and thus unable to survive long-distance shipping. Thus transport from Europe to Australia is more likely as hull-attached juvenile or adult worms than as larvae.

The indications are that the Mediterranean Sea is a more likely source of the Australian populations than the Atlantic Ocean. This is suggested both by the mean Nei genetic distance between the Mediterranean and the Australian collections being only 0.040 compared with 0.084 for the Atlantic and Australian collections, and by inspection of allele frequencies at individual loci. Of course, this conclusion bears the caveat that only one Atlantic sample, Roscoff, was examined, and it assumes that other Atlantic populations would be more like Roscoff than those from the Mediterranean.

Another issue concerns the likely number of introductions to Australia. In particular, do the three Australian populations represent the results of invasions from three different European source populations, or do they represent the results of one or more invasions from a single source population? The first scenario seems unlikely. There were no statistically significant differences in gene frequency among the Australian collections; the three populations were genetically homogeneous. Yet there was significant spatial heterogeneity observed among European populations. Introductions from spatially disjunct European populations would have led to genetic differences among the Australian populations. It therefore seems likely that there was a single European source population. Whether there were multiple introductions from that source, or a single introduction followed by a spread, cannot be readily determined. If the former, then the introductions would have to be of similar size and genetic composition; if the latter, then the secondary colonisations are likely to have involved appreciable numbers of individuals; small numbers would have led to some genetic divergence. The latter hypothesis of secondary colonisations seems somewhat more probable, as such introductions could perhaps be mediated not only by hull-borne individuals but also by transport of *Sabella* eggs and larvae in the ballast water of coastal shipping with short transit times. Thus while we cannot rule out other possibilities, we suggest that the genetic data are more consistent with a single introduction followed by a spread than with other scenarios.

If there was a single invasion of southern Australian waters, followed by a spread, then which area received that first invasion? The present genetic data do not answer that question, but Clapin and Evans (1995) suggest, from the examination of museum material, that *S. spallanzanii* may have been in the Albany region of Western Australia as early as 1965. This is presently the oldest record in Australia, and it is thus possible that while this is only a minor port, berthing only small and medium-sized vessels, this is the first port of introduction.

In principal, if there was a single introduction (say to Albany) followed by secondary colonisations from that original introduction, then the population resulting from the first colonisation should show more genetic variation than the second. If there was a linear sequence of colonisations, with the third introduction deriving from the second, and so on, then each subsequent introduction would be expected to result in the loss of further variability. The allozyme approach used here was not powerful enough to examine such expectations, since the numbers of alleles per locus, averaging 2 to 2.5, were too low. The analysis of microsatellite variation could well lead to very interesting results, since numbers of alleles per microsatellite locus can be as high as 40 or 50. With so many alleles, the consecutive loss of alleles with subsequent introductions should be measurable. The examination of five to ten such loci per species could well revolutionise our understanding of the routes followed by accidental introductions of both marine and terrestrial organisms.

7. ACKNOWLEDGEMENTS

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