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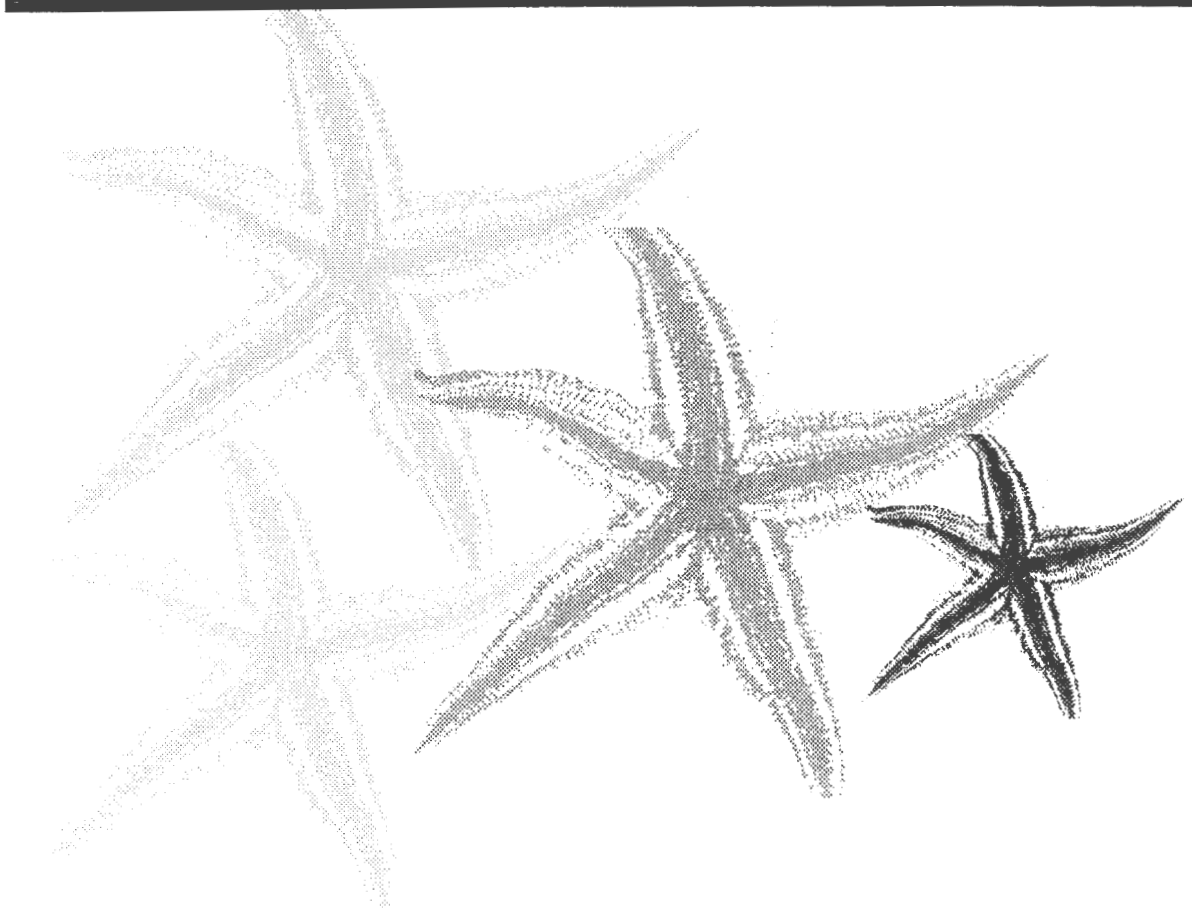
A PRELIMINARY ASSESSMENT OF THE GENETIC POPULATION STRUCTURE OF *ASTERIAS AMURENSIS* IN TASMANIAN, RUSSIAN, AND JAPANESE WATERS

PRINCIPAL INVESTIGATOR: R.D. WARD



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Ward, R.D. (Robert D.)
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CONTENTS

1. NON-TECHNICAL SUMMARY

The northern Pacific seastar, *Asterias amurensis*, has been recently introduced to Australian, specifically south-east Tasmanian, waters. It is endemic to the coasts of Japan and eastern Russia, and is also found along the Alaskan coast. Three populations from Tasmania (the Derwent River at Hobart, the D'Entrecasteaux Channel at Cygnet, and Triabunna) were examined genetically, and compared with six populations from Japan and one from Russia. Genetic variation was estimated by allozyme electrophoresis, and 22 loci were examined in all samples.

The Tasmanian populations had substantial levels of genetic variation, but these were lower than Japanese and Russian populations. It was estimated that in the process of colonization, about 36% of the genetic variation had been lost. This was probably lost during population size bottlenecks, associated with both a small number of original founders and a probable slow population size increase in the first few generations in Tasmania.

There were significant genetic differences among the populations from Japan and Russia. Three population groups were apparent: one group comprised three northern Japanese populations and the Russian population, the second group comprised two mid-Japan populations, and the third comprised the single population from southern Japan.

There were no significant genetic differences among the three Tasmanian samples. This suggests that there was a single introduction, and that animals have spread from the point of introduction. Alternatively, but less plausibly, there could have been several introductions from a single source population.

The Tasmanian animals were genetically much more similar to the mid-Japan populations than they were to the northern and southern Japanese populations or to the Russian population. Thus the introduction may have come from this area: it is unlikely to have come from southern or northern Japan or from the Russian population. However, there were some significant differences between the Tasmanian and mid-Japan populations which could be attributable to bottleneck effects or might mean that the source area has yet to be identified.

Future work to identify the possible source area should include the screening of additional overseas populations and the development of additional genetic markers. In addition, any future outbreaks in Australia should be examined genetically to identify their possible source.

2. BACKGROUND

The possibility of accidental introductions of exotic plant and animal species has been with us ever since man first travelled the seas. But over the last few decades, with the development of larger and faster ships, these risks have increased and in many instances been realised. Some of these introduced species have proliferated in their new environment and pose serious ecological and economic dangers. One such species is the northern Pacific seastar, *Asterias amurensis* Lutken, recently introduced to Tasmanian waters (Turner, 1992).

Asterias amurensis is a boreal species, inhabiting cold to temperate, sublittoral and shallow waters of the north and north-eastern areas of the Pacific Ocean. It is native to the coasts of eastern Russia and Japan and, while found through the Bering Sea to Alaska, it is uncertain whether the Alaskan populations are native or introduced. The species is morphologically extremely variable, expressing gradual variations between geographical populations (D'Yakanov, 1968; Onguru and Okutani, 1991). There appear to be six very similar subspecies, representing distinct morphological variants. One of these, the subspecies *Asterias amurensis versicolor* Sladen occurs in the warmer waters of south-western Japan (Nojima *et al.*, 1986). It has also been suggested that *Asterias amurensis* in Peter the Great Bay (off Vladivostok) comprises two closely related species (Manchenko, per. com. 1993), although a subsequent survey only revealed one species, *Asterias amurensis* (Manchenko, per. com. 1994).

This starfish is found at depths between 1 and 200m and has a preferred diet of shellfish such as mussels, scallops and clams. In Japan, sporadic outbreaks every ten or so years cause significant damage to natural and cultured shellfish beds (see, for example, Nojima *et al.* 1986). It is a dioecious species, although asexual reproduction can occur, following damage to the animal, by the regeneration of arms attached to a portion of the central disc. Females are highly fecund, spawning up to, and perhaps more than, 20 million eggs annually (Kasyanov, 1988). Under favourable conditions, population growth can be rapid. Free-swimming larvae develop following fertilisation. The final larval stage, the brachiolaria, attaches to a substrate and metamorphoses into a tiny starfish. The length of larval life is currently rather uncertain, although likely to be a minimum of six weeks and possibly substantially longer. Sagara and Ino (1954) cultured the bipinnaria (pre-settlement) larval stage over a 40 day period, Bruce (pers.com.) reared larvae for 7 weeks to the bipinnaria stage, Kasyanov (unpublished report) reared larvae for 7-8 weeks to the brachiolaria stage, and Komatsu (pers. com.) maintained larvae for 5 months without settlement. The high fecundity and long-lived planktonic larvae suggest that this species, once introduced, could rapidly colonise and spread throughout a new, biotically suitable, environment.

The first record of this starfish in Australian waters is a specimen taken from the Derwent River estuary at Hobart, Tasmania, and lodged with the Tasmanian Museum in October 1986. At the time it was incorrectly identified, and its true identity revealed only in March 1992. By then, the species was present in large numbers in the Derwent River (Turner, 1992). In July 1993, a team of 22 divers collected more than 6000 specimens in 3 hours from an area less than 300 by 20 metres off a Hobart wharf. On August 22, 1993, a more extensive dive around the Hobart wharves led to more than 24,000 animals being counted and to the removal of about three tonnes of the starfish (Johnson, 1994). It is now an important component of the shallow water fauna of the

Derwent River. For the present, its Australian distribution remains largely confined to the Derwent River and adjoining bays and channels, although there is a second apparently disjunct population at Triabunna on the east coast of Tasmania about 100 km north of Hobart. There is considerable concern among marine biologists and others that this exotic species will have an extremely serious impact on native ecosystems, and it represents a potential threat to the growing mariculture industry of Tasmania.

In May 1993, the CSIRO Division of Fisheries was commissioned by the Fisheries Research and Development Corporation to review known information on the introduced starfish, *Asterias amurensis*, and predict its potential impact on Australian fisheries and marine ecosystems. The conclusions, detailed in a status report produced in June (Davenport and McLoughlin, 1993), were that:

- *Asterias* colonised southern Tasmanian waters probably due to the introduction of larvae from ballast water.

- *Asterias* is an opportunistic feeder and will consume almost any animal tissue it can capture, but prefers mussels, scallops and clams.

- In its native environment, it has no significant natural predators, undergoes massive cyclic changes in population size, and, at high densities, has caused millions of dollars worth of damage to shellfish industries (Nojima *et al.*, 1986; Galtsoff and Loosanoff, 1939).

- Although the impact of *Asterias* in southern Australian waters cannot yet be stated with any certainty, the ecological plasticity of the species and the problems it presents in its natural environment are cause for grave concern. It is possible that *Asterias* could have a devastating impact on coastal wild fisheries (especially shellfish and crustacean fisheries), mariculture and the coastal marine ecosystem.

- *Asterias* larvae could spread from Tasmanian waters north into Bass Strait and hence, possibly, to mainland Australia.

The conclusion that the seastar entered Tasmanian waters via ballast water discharge from one or more overseas vessels is reasonable. Various other species are thought to have arrived here in a similar way, including the Japanese seaweed *Undaria* in the vicinity of Triabunna. Samples from Japanese seawater ballast taken in Australian ports were shown to include a wide variety of plankton including molluscs, crustaceans, urochordates and even two fish species (Williams *et al.*, 1988). An intensive analysis of Japanese seawater ballast released in Oregon, US, identified representatives of 367 taxa including asteroid echinoderms (Carlton and Geller, 1993). The release of freshwater ballast has been proposed as the source of various exotic species into the Great Lakes of Canada and the United States (Hebert *et al.*, 1989). Now that the seastar is well established, it may spread to other areas of Australia by any one of a number of mechanisms, including ballast water exchange, hitchhiking of larvae or adults in fish catches, attachment to ships' hulls or anchors, or by larval drift in ocean currents.

Genetic studies of this species appear to be restricted to the allozyme analysis of two *Asterias amurensis* populations, one from near Vladivostok, Russia (Manchenko, 1986), and one from Mutsu Bay, northern Japan (Matsuoka *et al.*, 1994). We decided to carry

out a similar allozyme analysis of various Tasmanian and overseas samples, rather than the possibly more powerful direct analysis of nuclear or mitochondrial DNA, because allozyme electrophoresis is quick, relatively cheap, and should provide sufficient data to answer the listed objectives (section 4).

3. NEED

The principal aim of the present project was to assess and to compare the extent of genetic variability in the introduced Tasmanian populations and in native Japanese and Russian populations. This was required for several reasons.

- Firstly, there is an immediate need to identify which subspecies is infesting Tasmanian waters, as this may determine its ultimate distribution. If it is the southern (warm water) subspecies, it is likely to be capable of invading Australian waters up to and including Sydney and Perth, while if it is a northern (cool water) subspecies, it may be restricted in terms of temperature tolerance to waters around Tasmania and Victoria.

- Secondly, genetic analysis will help us understand whether the three recorded outbreaks in Tasmania (the Derwent River, the d'Entrecasteaux Channel and Triabunna) represent three separate introductions or whether the species has spread from a single point introduction. This information bears directly on our understanding of both the dynamics of the introduced population and its likely rate of spread.

- Thirdly, this 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 could prove simpler to control a genetically monomorphic population than a polymorphic population. There have been relatively few studies which have compared the genetics of accidental rather than deliberate recent introductions with those of putative source populations. Some accidental introductions, for example the molluscs *Crepidula onyx* into Hong Kong, *C. fornicata* into Britain, and *Dreissena polymorpha* into North America, have been accompanied by the loss of relatively little genetic variation (Woodruff *et al.*, 1986; Hoagland, 1985; Boileau and Hebert, 1993), while others, such as *Littorina saxatilis* into South Africa (Knight *et al.*, 1987), have led to significant genetic erosion.

4. OBJECTIVES

To undertake a preliminary analysis of genetic variability in Tasmanian, Japanese and Russian populations of *Asterias amurensis* in order to:

- 1. Determine whether the introduced seastar is the southern sub-species, *A. amurensis versicolor*, or a northern subspecies.
- 2. Determine whether the Tasmanian populations are derived from one or several introductions.
- 3. Determine whether the invasion of Tasmanian waters has been accompanied by a loss of genetic variation with respect to native populations.

5. METHODS

5.1. COLLECTIONS

Seastars were collected from several locations:

Tasmania
Hobart (February 1994),
Triabunna (May 1994, from scallop spat collecting bags in the Mercury Passage, 60 km north-east of Hobart),
Cygnet, Deep Bay (July 1994, from the d'Entrecasteaux Channel, 50 km south of Hobart).

Japan (see Fig. 5.1)
Nemuro Bay (August 1993)
Yoichi (August 1993),
Mutsu Bay (October 1993, from Mutsu Bay, near Aomori),
Tokyo Bay (August 1993),
Suruga Bay (June, 1994),
Ariake Sea (August 1994)

Attempts to collect specimens from the Inland Sea of Japan (between the south of Honshu and the island of Shikoku) were unsuccessful. Apparently the species is very rare in this waterway (I. Imai, pers.com.).

Russia
Vladivostok (October 1993, Vostok Marine Field Station, about the same latitude as Yoichi).

Samples from Japan and Russia were air-freighted frozen to Hobart. At Hobart, all samples were maintained at -80^{o}C. Target sample sizes were 100 per site from Tasmania, and 50 per site from Japan and Russia. These target sample sizes were achieved (see Table 6.1), with two exceptions. Only 39 were collected from Yoichi, and animals were found to be relatively scarce in the d'Entrecasteaux Channel in Tasmania, but eventually six were collected from Deep Bay near Cygnet.

Fig. 5.1. Location of the six Japanese samples

5.2. ELECTROPHORETIC PROCEDURES

Extracts of pyloric caecae or tube legs 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 2 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 at 200V. Either a tris-glycine (Hebert and Beaton, 1989) or tris-citrate (75mM tris, 25mM citric acid, pH 7.0) buffer system was used. Table 5.1 lists the enzymes and experimental conditions used. When an enzyme is encoded by two loci, the more anodally migrating enzyme is suffixed as 1. Alleles are numbered according to the mobilities of their products relative to that of the most common allele in Tasmania, numbered 100. Interestingly, there was sufficient arginine phosphokinase enzyme present that it could be readily detected using a Coomassie blue general protein stain, in a similar fashion to fish muscle creatine kinase.

Several enzymes, including iditol (sorbitol) dehydrogenase and aldehyde oxidase, could not be routinely scored and therefore are not considered further.

5.3. 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. With very few exceptions, this meant that tests were carried out after pooling rarer alleles, producing two allele tests (with one degree of freedom). The exceptions were the loci *Xo,* where pooling to three alleles was acceptable, and *Pep-vl-1* and *Pgm-2* at Hobart and Triabunna, where the larger sample sizes meant that valid three-allele tests (with three degrees of freedom) could be deployed. The standard Bonferroni technique was used to adjust significance levels. The predetermined significance level of 0.05 was divided by the number of tests to obtain a corrected significance level.

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

1. Contingency chi-square tests were used to determine whether allele frequencies of variable loci were heterogeneous over samples. Where expected numbers per cell were 1 or greater, standard tests were used employing degrees of freedom. Where one or more cells had expected numbers less than one, the Monte Carlo randomisation approach of Roff and Bentzen (1989) was used. This eliminates the need to pool rare alleles. Two 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 2000 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 samples. G_{ST} is equal to $(H_T\text{-}H_S)/H_T$, where H_T (total genetic diversity) is the average of the Hardy-Weinberg expected heterozygosity in the total

population and H_S (mean genetic diversity per area) is the average Hardy-Weinberg expected heterozygosity within areas. 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 areas. 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 $G_{ST\text{-}null}$ and a mean value of $G_{ST\text{-}null}$ was estimated for each locus from 1000 replications. The number of times each of the 1000 estimates of $G_{ST\text{-}null}$ 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 samples 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 sample 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 dendrogram is a Wagner tree rooted at the mid-point of the longest genetic distance path. 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 sample size into account.

Table 5.1. Enzymes, loci, and routine tissue and electrophoresis conditions.

ENZYME	EC NUMBER	LOCUS	TISSUE	BUFFER	RUN TIME (MINS)
Malate dehydrogenase	1.1.1.37	*Mdh-1, Mdh-2*	tl	B	65
Isocitrate dehydrogenase	1.1.1.42	*Idh*	pc	B	50
Phosphogluconate dehydrogenase	1.1.1.44	*Pgdh*	pc	B	55
Octanol dehydrogenase	1.1.1.73	*Odh*	pc	A	20
Diaphorase	1.8.1.4	*Dia-1, Dia-2*	tl	B	60
Xanthine oxidase	1.2.3.2	*Xo*	pc	A	25
Aspartate aminotransferase	2.6.1.1	*Aat-1*	pc	A	25
Hexokinase	2.7.1.1	*Hk*	tl	B	60
Arginine phosphokinase	2.7.3.3	*Apk*	tl	A	25
Nucleoside diphosphate kinase	2.7.4.6.	*Ndpk*	pc	B	60
Esterase D	3.1.1.-	*Est-D-1, Est-D-2*	tl	A	15
Peptidase (val-leu)	3.4.11.-	*Pep-vl-1, Pep-vl-2*	tl	B	60
Peptidase (leu-pro)	3.4.11.-	*Pep-lp-1, Pep-lp-2*	tl	A	23
Leucine aminopeptidase*	3.4.11.1	*Lap-1, Lap-2*	pc	A	18
Mannosephosphate isomerase	5.3.1.8	*Mpi*	pc	A	18
Glucosephosphate isomerase	5.3.1.9	*Gpi*	tl	B	90
Phosphoglucomutase	5.4.2.2.	*Pgm-1, Pgm-2*	tl	A	25

pc=pyloric caecae, tl=tube legs, A=tris glycine system, B=tris citrate system
* This enzyme did not appear in samples from the Ariake Sea.

6. DETAILED RESULTS

6.1 OVERALL DESCRIPTION OF GENETIC VARIABILITY

One enzyme, leucine aminopeptidase, was not apparent in the sample from the Ariake Sea. The reason for this is not known, since all other enzymes were fully active in this sample. In all other samples, it was encoded by two monomorphic loci, *Lap-1* and *Lap-2*. Table 6.1 gives allele frequencies and locus heterozygosities. Since the *Lap* loci could not be scored in the Ariake Sea specimens, these loci are not considered further. Table 6.2 summarises data on genetic variation in each sample for the 22 loci scored in each sample.

Genotype numbers in most samples accorded with Hardy-Weinberg expectations (Table 6.3). A total of 64 valid tests was performed, with just eight showing a significant departure from expectation. Most of these significant results were only just significant, with probability values between 0.05 and 0.01. The two most divergent samples were *Hk* at Yoichi (P=0.009) and *Pep-lp-2* at Nemuro Bay (P<0.001). After Bonferroni adjustment (with the significance level being reduced from 0.05 to 0.05/64 or 0.001), only one sample remained significant (*Pep-lp-2* at Nemuro Bay).

There was no evidence for a consistent excess or deficiency of heterozygotes. Of the eight tests with P<0.05, four showed a heterozygote excess, four a heterozygote deficiency. These generally good fits to Hardy-Weinberg equilibrium indicate that this is a sexually reproducing species, with little recruitment from asexually produced animals derived from the regeneration of broken arms. However, with sample sizes in the range of 40-100, Hardy-Weinberg tests will not be of sufficient power to detect asexual recruitment at low levels.

The three Tasmanian samples were clearly less variable, over all loci, than the Japanese and Russian samples (Table 6.2). Mean numbers of alleles per locus were around 1.5 in the Tasmanian sample, compared with between 2 and 2.4 in the overseas samples. The average sample size for the overseas samples was about half that of the Derwent and Triabunna samples: had the overseas sample sizes been larger, it is possible that this difference would have been accentuated. The mean percentage of loci polymorphic in the Tasmanian samples ranged from 22.7 to 27.3 (0.95 criterion) and 22.7 to 31.8 (0.99 criterion), values again appreciably less than in the overseas samples, 40.9 to 45.5 (0.95) and 45.5 to 54.6 (0.99). The best single parameter for comparing variation is mean 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 Tasmanian samples ranges from 0.116 to 0.127, compared with 0.177 to 0.216 for the overseas samples.

This reduction in genetic variability in the Tasmanian samples essentially arises from these samples being monomorphic at four loci (*Gpi, Hk, Pep-lp-2, Pep -vl-2)* that were highly polymorphic in all overseas populations. However, one locus shows the reverse trend. This is *Mdh-1*, a locus showing substantial variation in Tasmania (average heterozygosity per population around 0.40), but only low levels of variation overseas (average heterozygosity per population 0 to 0.08).

Table 6.1. Allele frequencies, sample sizes (N=number of individuals sampled), and locus heterozygosities (het=Hardy Weinberg expected heterozygosity).

Locus	allele	Derwent	Triabunna	Cygnet	Ariake Sea	Suruga Bay	Tokyo Bay	Mutsu Bay	Yoichi	Nemuro Bay	V'vostok
Variable loci											
Mdh-1	110	0	0	0	0.026	0.030	0.020	0.030	0.013	0.019	0
	100	0.695	0.765	0.833	0.974	0.970	0.970	0.960	0.987	0.982	1
	90	0.305	0.235	0.167	0	0	0.010	0.010	0	0	0
	N	100	100	6	58	50	50	50	39	54	50
	het	0.424	0.360	0.278	0.050	0.058	0.059	0.077	0.025	0.036	0
Mdh-2	100	1	1	1	1	1	1	1	1	0.991	0.980
	90	0	0	0	0	0	0	0	0	0.009	0.020
	N	100	100	6	58	50	50	50	39	54	50
	het	0	0	0	0	0	0	0	0	0.018	0.039
Idh	110	0	0	0	0	0	0.010	0	0	0	0
	100	0.990	1	1	0.897	1	0.990	0.980	1	1	1
	90	0.010	0	0	0.103	0	0	0.020	0	0	0
	N	92	99	6	58	50	50	50	38	54	50
	het	0.020	0	0	0.185	0	0.020	0.039	0	0	0
Pgdh	120	0	0	0	0.026	0	0.030	0.010	0	0.009	0.050
	110	0.500	0.450	0.500	0.767	0.530	0.570	0.490	0.411	0.453	0.540
	100	0.500	0.550	0.500	0.207	0.470	0.400	0.500	0.589	0.528	0.410
	90	0	0	0	0	0	0	0	0	0.009	0
	N	97	100	6	58	50	50	50	28	53	50
	het	0.500	0.495	0.500	0.368	0.498	0.514	0.510	0.484	0.516	0.538
Dia-1	100	1	1	1	1	0.990	1	1	1	0.981	0.974
	90	0	0	0	0	0.010	0	0	0	0.019	0.026
	N	100	55	6	58	50	50	47	32	54	39
	het	0	0	0	0	0.020	0	0	0	0.037	0.051
Xo	125	0	0	0	0	0	0	0	0	0.010	0
	115	0.125	0.095	0	0.388	0.207	0.318	0.319	0.425	0.330	0.359
	100	0.730	0.715	0.750	0.371	0.587	0.546	0.489	0.425	0.400	0.402
	85	0.130	0.165	0.250	0.164	0.185	0.125	0.149	0.138	0.230	0.228
	70	0.015	0.025	0	0.078	0.022	0.011	0.043	0.013	0.030	0.011
	N	100	100	6	58	46	44	47	40	50	46
	het	0.434	0.452	0.375	0.679	0.578	0.585	0.635	0.620	0.677	0.657

Table 6.1 continued.

Locus	allele	Derwent	Triabunna	Cygnet	Ariake Sea	Suruga Bay	Tokyo Bay	Mutsu Bay	Yoichi	Nemuro Bay	V'vostok
Aat	130	0	0	0	0.017	0.010	0.010	0	0	0	0.010
	100	1	1	1	0.983	0.990	0.990	1	1	0.988	0.990
	85	0	0	0	0	0	0	0	0	0.012	0
	N	100	100	6	58	50	50	50	18	43	50
	het	0	0	0	0.034	0.020	0.020	0	0	0.024	0.020
Hk	100	1	1	1	0.802	0.570	0.530	0.500	0.423	0.500	0.270
	90	0	0	0	0.198	0.400	0.470	0.480	0.577	0.491	0.730
	85	0	0	0	0	0.030	0	0.020	0	0.009	0
	N	100	100	6	58	50	50	50	39	54	50
	het	0	0	0	0.318	0.514	0.498	0.519	0.488	0.509	0.394
Apk	100	0.930	0.905	1	0.216	0.880	0.920	0.080	0.026	0	0.050
	90	0.070	0.095	0	0.690	0.110	0.070	0.210	0.128	0.037	0.570
	80	0	0	0	0.086	0.010	0.010	0.690	0.782	0.843	0.380
	70	0	0	0	0.009	0	0	0.020	0.064	0.120	0
	N	100	100	6	58	50	50	50	39	54	50
	het	0.130	0.172	0	0.470	0.213	0.133	0.473	0.367	0.274	0.528
Est-D-2	110	0	0	0	0.035	0	0	0	0	0	0
	100	1	1	1	0.966	1	1	1	1	1	1
	N	100	100	6	58	50	50	49	28	54	34
	het	0	0	0	0.067	0	0	0	0	0	0
Pep-vl-1	110	0.265	0.220	0.167	0.009	0.030	0.030	0	0.013	0.019	0.010
	100	0.475	0.435	0.417	0.517	0.460	0.460	0.390	0.628	0.519	0.470
	90	0.260	0.345	0.417	0.474	0.480	0.440	0.580	0.321	0.417	0.480
	80	0	0	0	0	0.030	0.070	0.030	0.039	0.046	0.040
	N	100	100	6	58	50	50	50	39	54	50
	het	0.637	0.643	0.625	0.508	0.556	0.589	0.511	0.501	0.555	0.547
Pep-vl-2	140	0	0	0	0	0	0.030	0	0	0.009	0
	120	0	0	0	0.164	0.316	0.380	0.449	0.276	0.130	0.240
	100	1	1	1	0.836	0.684	0.590	0.551	0.724	0.861	0.760
	N	100	100	6	58	49	50	49	38	54	50
	het	0	0	0	0.274	0.433	0.507	0.495	0.400	0.242	0.365

Locus	allele	Derwent	Triabunna	Cygnet	Ariake Sea	Suruga Bay	Tokyo Bay	Mutsu Bay	Yoichi	Nemuro Bay	V'vostok
Pep-lp-2	120	0	0	0	0	0.030	0	0.060	0	0	0
	110	0	0	0	0.061	0.220	0.110	0.200	0.129	0.123	0.160
	100	1	1	1	0.912	0.740	0.870	0.620	0.800	0.717	0.560
	90	0	0	0	0.026	0.010	0.020	0.090	0.057	0.142	0.260
	80	0	0	0	0	0	0	0.030	0.014	0.019	0.020
	N	100	100	6	57	50	50	50	35	53	50
	het	0	0	0	0.163	0.403	0.231	0.563	0.340	0.451	0.593
Gpi	180	0	0	0	0	0.010	0	0.030	0.013	0.009	0
	150	0	0	0	0.043	0.020	0.020	0.040	0.038	0	0.010
	130	0	0	0	0	0.080	0.060	0.190	0.052	0.102	0.090
	100	1	1	1	0.922	0.880	0.920	0.740	0.897	0.880	0.890
	70	0	0	0	0.035	0.010	0	0	0	0.009	0.010
	N	100	100	6	58	50	50	50	39	54	50
	het	0	0	0	0.146	0.219	0.150	0.414	0.190	0.216	0.200
Pgm-2	200	0	0	0	0.009	0	0	0	0	0	0.030
	180	0.180	0.165	0.083	0.138	0.071	0.120	0.070	0.103	0.148	0.030
	160	0	0	0	0.216	0.122	0.100	0.050	0.064	0.120	0.180
	140	0.355	0.375	0.417	0.586	0.633	0.520	0.720	0.731	0.602	0.470
	120	0	0	0	0.026	0.051	0.110	0.070	0.051	0.083	0.200
	100	0.465	0.460	0.500	0.026	0.112	0.150	0.090	0.051	0.046	0.070
	80	0	0	0	0	0.010	0	0	0	0	0.020
	N	100	100	6	58	49	50	50	39	54	
	het	0.625	0.621	0.569	0.589	0.564	0.671	0.461	0.446	0.592	0.700
Invariant loci (all with 100 allele)											
Dia-2	N	100	55	6	50	48	50	49	32	54	39
Odh	N	94	93	6	50	44	50	50	39	42	38
Ndpk	N	40	56	6	35	33	32	28	34	49	50
Est-D-1	N	100	100	6	50	50	50	49	39	54	39
Pep-lp-1	N	100	88	6	50	39	50	50	39	54	50
Lap-1	N	90	55	6	*nd*	40	40	40	35	40	40
Lap-2	N	90	55	6	*nd*	40	40	40	35	40	40
Mpi	N	97	100	6	50	50	50	39	39	54	50
Pgm-1	N	93	35	6	35	30	30	38	38	20	34

nd = not detected, and so these two loci were not used in any subsequent statistical analyses

Table 6.2. Summary of genetic variability in all samples (± standard errors) for the 22 common loci.

Sample	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic* 0.95	0.99	Mean HW expected heterozygosity per locus
Derwent	96.0 ± 2.7	1.5 ± 0.2	27.27	31.82	0.127 ± 0.049
Triabunna	90.0 ± 4.2	1.5 ± 0.2	27.27	27.27	0.125 ± 0.048
Cygnet	6.0 ± 0.0	1.3 ± 0.1	22.73	22.73	0.116 ± 0.049
Ariake Sea	54.0 ± 1.5	2.1 ± 0.3	45.45	59.09	0.177 ± 0.047
Suruga Bay	47.2 ± 1.2	2.2 ± 0.3	40.91	54.55	0.187 ± 0.051
Tokyo Bay	48.0 ± 1.2	2.1 ± 0.3	40.91	54.55	0.183 ± 0.053
Mutsu Bay	47.5 ± 1.2	2.2 ± 0.3	40.91	50.00	0.216 ± 0.055
Yoichi	35.9 ± 1.2	2.0 ± 0.3	40.91	45.45	0.178 ± 0.049
Nemuro	50.9 ± 1.6	2.4 ± 0.3	40.91	54.55	0.190 ± 0.052
Vladivostok	46.3 ± 1.3	2.2 ± 0.3	40.91	54.55	0.213 ± 0.058

*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

Table 6.3. Summary of Hardy-Weinberg tests for polymorphic loci. Cygnet data ignored as sample size was too small for valid testing.

P: Probabilities that samples accord with Hardy-Weinberg expectations. Only probabilities less than 0.05 are given; non-significant values of the chi-square statistic are given as ns.
- denotes either the locus was monomorphic or insufficient variation for a statistically valid test.
D: The direction of deviation from Hardy-Weinberg expectations. Values of D can in principle range from -1 (no heterozygotes observed) to +1 (only heterozygotes observed), so negative values indicate a deficiency of heterozygotes, positive values an excess.

Population		*Mdh-1*	*Pgdh*	*Xo*	*Hk*	*Apk*	*Pep-vl-1*	*Pep-vl-2*	*Pep-lp-2*	*Gpi*	*Pgm-2*
Hobart	*P*	ns	ns	ns	-	-	ns	-	-	-	ns
	D	0.109	0.175	-0.001			-0.010				0.007
Triabunna	*P*	ns	ns	ns	-	-	0.017	-	-	-	0.017
	D	0.085	0.091	-0.038			-0.161				0.080
Ariake Sea	*P*	-	ns	ns	ns	ns	ns	ns	-	-	ns
	D		0.207	-0.156	0.247	0.047	0.196	-0.182			-0.076
Suruga Bay	*P*	-	ns	ns	ns	-	ns	0.041	ns	-	0.026
	D		0.004	-0.047	0.102		-0.119	-0.292	0.144		0.317
Tokyo Bay	*P*	-	ns	ns	0.022	-	ns	ns	ns	-	ns
	D		0.020	-0.142	0.325		0.109	-0.215	-0.027		0.042
Mutsu Bay	*P*	-	ns	ns	ns	ns	ns	ns	ns	ns	ns
	D		-0.061	-0.111	-0.040	-0.018	-0.097	-0.093	-0.151	0.040	0.091
Yoichi	*P*	-	ns	ns	0.009	ns	ns	0.012	ns	-	ns
	D		-0.041	-0.108	0.418	0.128	-0.177	-0.408	0.071		-0.283
Nemuro Bay	*P*	-	ns	ns	ns	-	ns	ns	<0.001	-	ns
	D		0.060	-0.088	-0.111		0.039	0.007	-0.535		0.043
Vladivostok	*P*	-	ns	ns	ns	ns	ns	ns	ns	ns	ns
	D		-0.114	-0.134	-0.138	0.102	-0.039	-0.013	0.055	-0.081	-0.077

6.2 COMPARING THE THREE TASMANIAN SAMPLES AGAINST ONE ANOTHER

Target sample sizes from the Tasmanian samples were larger than for the overseas samples. This was to increase the statistical power of tests aimed at examining whether the Tasmanian animals came from one or several introductions. Large samples were collected from the Derwent River and from Triabunna, but a paucity of animals in the D'Entrecasteaux Channel led to only six animals being collected from this area (Deep Bay, Cygnet).

There were no significant differences in gene frequency among these three samples at any of the six polymorphic loci (Table 6.4). Nor were there any significant differences when the two large samples alone (Derwent River and Triabunna) were compared against one another. Indeed, none of the statistical comparisons came close to demonstrating any spatial heterogeneity in gene frequencies.

Table 6.4. Tests of genetic spatial heterogeneity among Tasmanian samples.

Standard chi-square tests were used except for Xo for all three populations, where Monte-Carlo randomisation tests were deployed with 2000 replications per test.

Locus	All three populations			Derwent versus Triabunna		
	$\chi 2$	df	*P*	$\chi 2$	df	*P*
Mdh-1	3.137	2	0.208	2.481	1	0.115
Pgdh-1	1.017	2	0.601	0.987	1	0.320
Apk	1.924	2	0.380	0.826	1	0.364
Pep-vl-1	4.488	4	0.344	3.680	2	0.159
Pgm-2	0.912	4	0.923	0.245	2	0.885
Xo	4.588	(2000 tests)	0.560	2.180	3	0.536

6.3 COMPARING THE OVERSEAS SAMPLES AGAINST ONE ANOTHER

Fifteen loci showed variation in the seven overseas populations. Ten of these loci were polymorphic (definition of polymorphism: frequency of the most common allele no greater than 0.95) in at least one population and nine were polymorphic in all seven populations. Many variable loci showed spatial variation in allele frequencies (Table 6.5). After adjusting probability values to allow for multiple tests (P for significance at the 0.05 level decreasing from 0.05 to 0.05/15=0.0033), eight loci showed significant spatial heterogeneity following both contingency chi-square and bootstrapping G_{ST} analysis.

Over all loci, the 'true' G_{ST} (estimated from the observed value of G_{ST} minus the quantity $G_{ST.null}$ resulting from sampling error) was 0.096 (Table 6.5). This means that about 10% of the total genetic variation at this locus results from differences between populations, with about 90% coming from within-population variation. This differentiation was not spread equally among loci. Some loci (eg *Pep-vl-1*) showed no significant spatial heterogeneity in allele frequencies, while the most striking heterogeneity was apparent for *Apk*. Here the 'true' G_{ST} was a high 0.468, meaning that about 47% of the total genetic variation at this locus results from differences between populations, with about 53% coming from within-population variation. Pairwise comparisons of allele frequencies of the seven samples showed that only two pairs of samples showed non-significant differentiation at the *Apk* locus. These were Suruga Bay/Tokyo Bay (P=0.613) and Mutsu Bay/Yoichi (P=0.083). The next highest 'true' G_{ST} value was nearly an order of magnitude lower, at 0.058 for the *Idh* locus.

The two most similar samples overall were Tokyo Bay and Suruga Bay, which were separated by only about 150 km. These showed small allele frequency differences for but one locus, *Pep-lp-2* (P=0.045), and after allowing for multiple tests even this one comparison becomes non-significant.

Table 6.5. Tests of genetic spatial heterogeneity among overseas samples.

Given the small expected values of some rare allele cells, Monte-Carlo randomisation chi-square tests were deployed, with 2000 replications per test.[1]

Locus	Chi-square analysis			Genetic diversity analysis*			
	$\chi 2$	*P*		*GST*	*GST.null*	'true' *GST*	*P*
Mdh-1	8.547	ns		0.006	0.009	0	ns
Mdh-2	8.563	ns		0.013	0.009	0.004	ns
Idh	57.060	<0.001		0.067	0.009	0.058	<0.001
Pgdh	50.717	<0.001		0.048	0.009	0.039	<0.001
Dia-1	8.039	ns		0.013	0.009	0.004	ns
Xo	39.17	0.018		0.018	0.009	0.009	0.017
Aat	9.666	ns		0.005	0.010	0	ns
Hk	77.762	<0.001		0.088	0.009	0.079	<0.001
Apk	736.993	<0.001		0.477	0.009	0.468	<0.001
Est-D-2	18.390	0.009		0.030	0.010	0.020	0.007
Pep-vl-1	25.983	ns		0.019	0.009	0.010	0.021
Pep-vl-2	54.500	<0.001		0.056	0.009	0.047	<0.001
Pep-lp-2	115.403	<0.001		0.058	0.009	0.049	<0.001
Gpi	53.798	<0.001		0.030	0.009	0.021	<0.001
Pgm-2	97.788	<0.001		0.029	0.009	0.020	<0.001
			overall	0.105	0.009	0.096	<0.001

*[1] *'True' GST = GST - GST.null*

6.4 GENETIC SIMILARITIES BETWEEN THE TASMANIAN AND OVERSEAS SAMPLES

Matrices of genetic distance and identity between all pairwise sample comparisons are given in Table 6.6 (Nei's 1978 measures) and Table 6.7 (Rogers 1972 measures). A UPGMA tree derived from Nei's distances is given in Fig. 6.1 and a Wagner tree from Rogers distances in Fig. 6.2.

The UPGMA-derived dendrogram of Nei's (1978) genetic distance matrix (Fig. 6.1) shows a number of interesting features:

1. The three Tasmanian samples are, as expected from the earlier analyses (section 6.2), very closely related to one another.

2. The dendrogram has a single major split, with the three Tasmanian samples falling onto the same major lineage as the Suruga Bay and Tokyo Bay samples. The latter two samples cluster together and come from adjacent locations on the main island of Japan, Honshu.

3. The northern Japanese samples cluster together, and group with the Vladivostok sample.

4. The southern sample, Ariake Bay, *A. amurensis versicolor*, clusters loosely with the northern samples: it does not form a lineage separated from all other lineages.

This pattern of relationships is repeated very closely in the Wagner tree of Rogers (1972) distance (Fig 6.2), although there is a small difference in the way the three northern Japanese samples cluster. This degree of consistency, despite the differences in ways of calculating genetic distance and different underlying assumptions of UPGMA and Wagner trees (section 5.3), suggests that these population groupings are likely to reflect real affinities. Nevertheless, the screening of additional loci would be useful to confirm these putative relationships.

Table 6.6. Nei's (1978) unbiased genetic distances (below diagonal) and genetic identities (above diagonal) between all pairs of samples for all 22 loci

	Hobart	Triabunna	Cygnet	Ariake Sea	Suruga Bay	Tokyo Bay	Mutsu Bay	Yaichi	Nemuro	V'vostok
Hobart	-	1.000	1.000	0.948	0.969	0.967	0.917	0.918	0.923	0.910
Triabunna	0.000	-	1.000	0.951	0.973	0.970	0.923	0.923	0.927	0.915
Cygnet	0.000	0.000	-	0.950	0.979	0.975	0.924	0.922	0.928	0.914
Ariake Sea	0.054	0.050	0.051	-	0.967	0.965	0.963	0.959	0.959	0.972
Suruga Bay	0.031	0.028	0.021	0.033	-	0.999	0.966	0.959	0.955	0.961
Tokyo Bay	0.033	0.031	0.025	0.036	0.001	-	0.962	0.957	0.951	0.958
Mutsu Bay	0.087	0.080	0.079	0.038	0.034	0.039	-	0.993	0.991	0.984
Yoichi	0.085	0.081	0.082	0.042	0.042	0.044	0.007	-	0.998	0.982
Nemuro	0.081	0.076	0.075	0.042	0.046	0.050	0.009	0.002	-	0.981
V'vostok	0.095	0.089	0.090	0.029	0.040	0.042	0.016	0.018	0.020	-

Table 6.7 Rogers (1972) genetic distances (below diagonal) and genetic identities (above diagonal) between all pairs of samples for all 22 loci

	Habart	Triabunna	Cygnet	Arioke Sea	Suruga Bay	Tokyo Bay	Mutsu Bay	Yoichi	Nemuro	V'vostok
Hobart	-	0.987	0.974	0.871	0.902	0.903	0.842	0.851	0.858	0.838
Triabunna	0.013	-	0.980	0.875	0.907	0.905	0.848	0.858	0.866	0.844
Cygnet	0.026	0.020	-	0.875	0.908	0.906	0.849	0.853	0.864	0.844
Ariake Sea	0.129	0.125	0.125	-	0.909	0.911	0.892	0.901	0.908	0.911
Suruga Bay	0.098	0.093	0.092	0.091	-	0.970	0.929	0.921	0.924	0.917
Tokyo Bay	0.097	0.095	0.094	0.089	0.030	-	0.924	0.918	0.918	0.914
Mutsu Bay	0.158	0.152	0.151	0.108	0.071	0.076	-	0.947	0.945	0.925
Yoichi	0.149	0.142	0.147	0.099	0.079	0.082	0.053	-	0.961	0.928
Nemuro	0.142	0.134	0.136	0.092	0.076	0.082	0.055	0.039	-	0.936
V'vostok	0.162	0.156	0.156	0.089	0.083	0.096	0.075	0.072	0.064	-

Figure 6.1 UPGMA tree of Nei's (1978) distance. 22 loci.

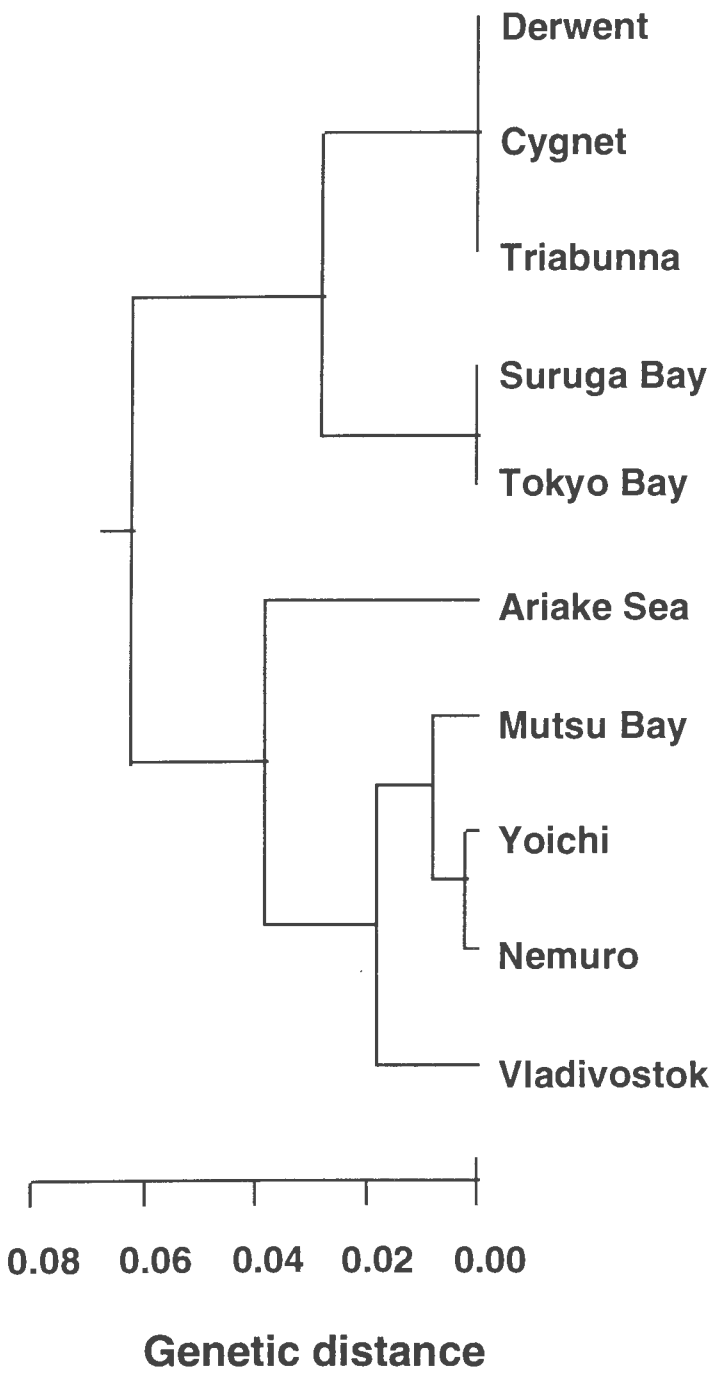
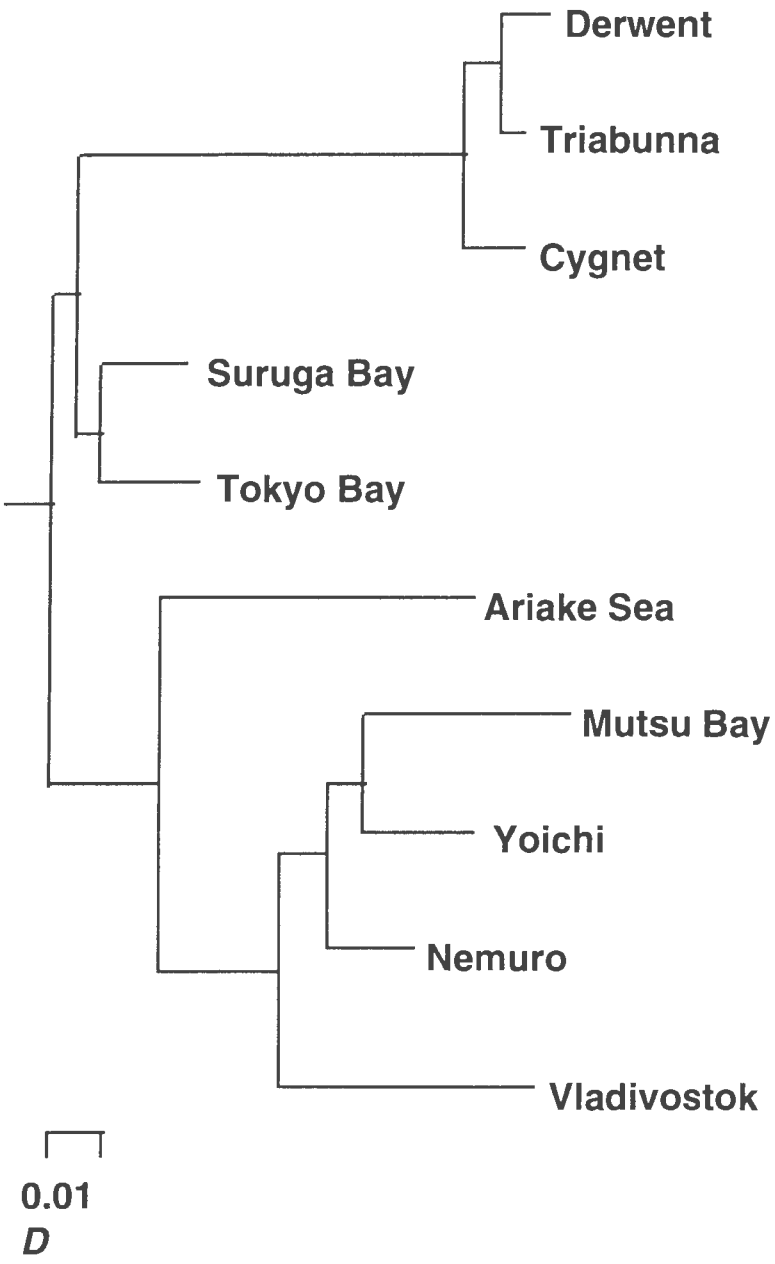


Fig. 6.2. Wagner tree of Rogers distances (*D*), rooted at mid-point of longest path. 22 loci.



One locus, *Apk*, shows far more inter-sample differentiation than any other locus (section 6.3), and therefore contributes most to the genetic distance differences of Figs 6.1 and 6.2. A matrix of genetic distances among samples for this locus alone is given in Table 6.8, and the derived Nei-UPGMA and Rogers-Wagner trees in Figs 6.3 and 6.4. The Cygnet population had to be dropped from the Nei-UPGMA tree as its Nei distance of infinity to the Nemuro population (no shared alleles) posed insoluble problems to the clustering algorithm. It could be included in the Rogers-Wagner tree as Rogers distance takes a range of 0 to 1 rather than 0 to infinity. These trees show the same general relationships as the trees using all 22 loci, except that the similarities among the Tasmanian and Suruga Bay/Tokyo Bay samples are emphasised, and the Russian population separates more clearly from the northern Japanese populations. Furthermore, the position of the Ariake Sea sample varies in the two dendrograms, clustering closely with the Vladivostok sample in the UPGMA tree but not the Wagner tree. At the *Apk* locus, the Tasmanian and Suruga Bay/Tokyo Bay samples show gene frequencies that are effectively identical: all these samples have allele frequencies of around 0.90 for *Apk*100 and around 0.10 for *Apk*90.

Table 6.8 Nei's (1978) genetic distances (below diagonal) and Rogers (1972) genetic distances (above diagonal) between all pairs of samples for the Apk locus alone.

	Hobart	Triabunna	Cygnet	Ariake Sea	Surugo Bay	Tokyo Bay	Mutsu Bay	Yoichi	Nemuro	V'vostok
Hobart	-	0.025	0.070	0.672	0.046	0.010	0.781	0.848	0.892	0.764
Triabunna	0.000	-	0.095	0.647	0.022	0.022	0.765	0.834	0.879	0.742
Cygnet	0.002	0.005	-	0.741	0.115	0.075	0.827	0.889	0.929	0.828
Ariake Sea	1.000	0.928	1.214	-	0.626	0.666	0.554	0.648	0.727	0.253
Suruga Bay	0.000	0.000	0.006	0.880	-	0.040	0.746	0.816	0.862	0.720
Tokyo Bay	0.000	0.000	0.002	0.994	0.000	-	0.771	0.838	0.882	0.756
Mutsu Bay	2.023	1.962	2.198	0.860	1.852	1.946	-	0.100	0.187	0.337
Yoichi	3.114	3.014	3.442	1.266	2.759	2.895	0.005	-	0.089	0.425
Nemuro	5.723	5.393	infinity	1.827	4.099	4.265	0.038	0.004	-	0.507
V'vostok	1.997	1.832	2.617	0.126	1.700	1.949	0.246	0.376	0.532	-

Figure 6.3 UPGMA tree of Nei's (1978) distance. *Apk* locus only. Cygnet population excluded (see text).

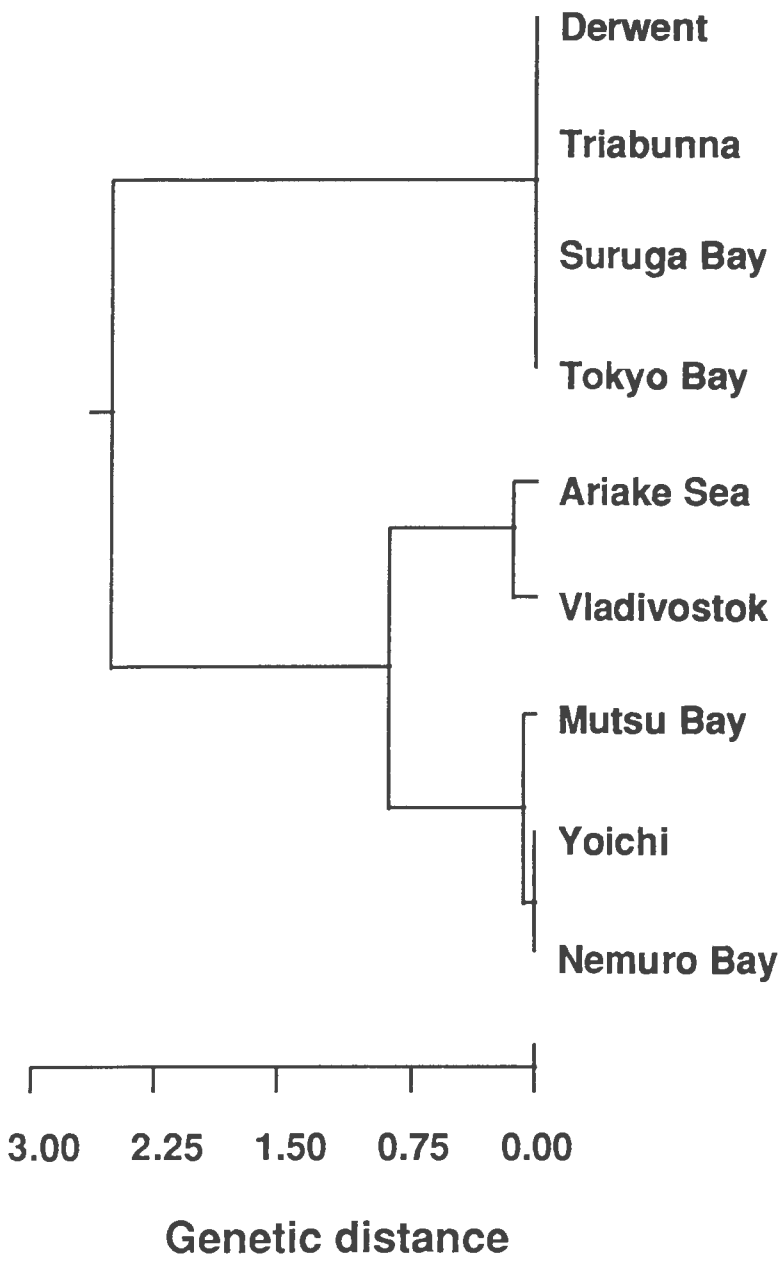
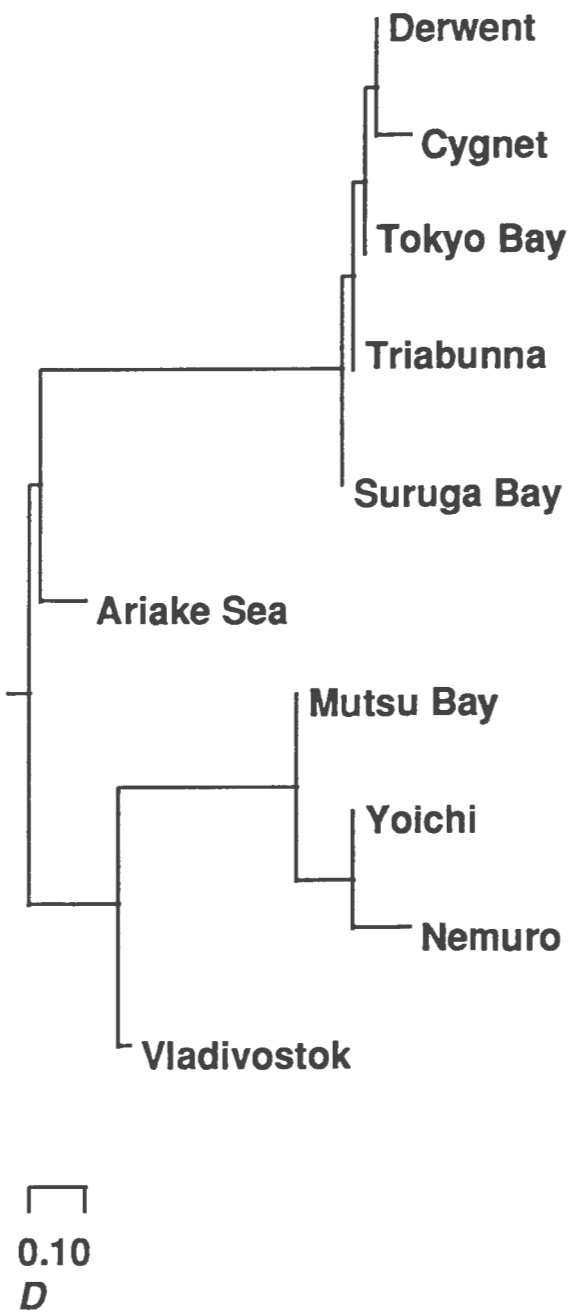


Figure 6.4. Wagner tree of Rogers distances (*D*), rooted at midpoint of longest path. *Apk* locus only.



6.5. DISCUSSION

The achievements of this project generally exceed the performance indicators listed in the proposal. In the proposal, we aimed to assess three Japanese populations for 50 starfish each, whereas in fact we examined six populations, with an average sample size of 50.2. This sampling effort was quite considerable, given the geographic and cultural separation between Australia and Japan, and allowed a more powerful than anticipated examination of the relationships between the introduced and Japanese samples. One area where we did not quite meet the performance indicators was in analysing introduced Tasmanian populations: instead of analysing three such samples for 100 specimens each, we managed 100 specimens for only two of the samples, the third sample having a sample size of six. This was beacuse of difficulties in collecting a sample from the d'Entrecasteaux Channel, where animals were apparently scarcer than we had anticipated. We met the target of analysing one Russian sample of 50 specimens. Furthermore, in the proposal we anticipated being able to screen 15-20 allozyme loci; in the event we satisfactorily analysed 22 loci in all samples.

The three major objectives of this study were to:

- 1. Determine whether the introduced seastar is the southern Japanese sub-species, *A. amurensis versicolor*, or a northern subspecies.
- 2. Determine whether the Tasmanian populations are derived from one or several introductions.
- 3. Determine whether the invasion of Tasmanian waters has been accompanied by a loss of genetic variation with respect to native populations.

These objectives will be discussed in turn.

- 1. Determine whether the introduced seastar is the southern Japanese sub-species, *A. amurensis versicolor*, or a northern subspecies.

A sample of *Asterias amurensis versicolor* was collected from the Ariake Sea on the southern Japanese island of Kyushu. Colour-wise, these animals looked quite different from the Tasmanian starfish and those from more northern parts of Japan and Russia. All the Ariake Sea specimens had a uniform deep purple to black upper surface with a pale line running down the middle of each arm, a pigmentation pattern that was absent from all other samples. The other samples showed a range of colours, varying from pale yellow to deep purple, and many specimens showed dark tips to their arms. Thus, purely on morphological grounds, it looked unlikely that the Tasmanian specimens were *A. amurensis versicolor*. But while pigment patterns are often genetically determined, in some animals they are known to reflect environmental or dietary conditions, and so this observation by itself is not definitive: it is possible that some special condition of the Ariake Sea, such as warmer temperatures, lead to this pigment pattern which could change under cooler conditions.

However, the genetic data also show that the Tasmanian animals are unlikely to be *A. amurensis versicolor*. If they were indeed that subspecies, then they would be expected to cluster with the sample of that subspecies in the multi-locus genetic dendrograms (Figs 6.1 and 6.2). Instead the Tasmanian animals do not cluster with the Ariake Sea

sample but rather with the Tokyo Bay and Suruga Bay samples. The bulk of the differentiation among samples is attributable to the highly differentiated *Apk* locus (G_{ST}= 47% among the overseas samples). At this locus, the Tasmanian samples cluster very tightly with the Suruga Bay/Tokyo Bay samples, and are well separated from all other samples including that from the Ariake Sea (Figs 6.3 and 6.4). The Tasmanian/Suruga Bay/Tokyo Bay animals are the only samples that show a high frequency of the *Apk*100 allele (about 0.90). This allele is very rare in the northern Japanese and Russian samples, and at low frequency in the Ariake Sea (about 0.20). The Tasmanian animals are unlikely to have come from northern Japanese regions. Not only did these three northern samples have low frequencies of the *Apk* 100 allele, they all had high frequencies (0.70-0.85) of the *Apk* 80 allele, an allele not observed in the Tasmanian animals.

Of all the Japanese and Russian samples analysed, the Tasmanian samples are clearly most closely related to the Suruga Bay/Tokyo Bay samples. These latter two samples are very similar to each other. But this does not necessarily mean that the Tasmanian animals came from one of these two bays. There might be neighbouring (or even distant) bays with similar genetic constitutions. Furthermore, there are some significant differences between the Tasmanian and Suruga Bay/Tokyo Bay samples. Not only has variation been lost at some loci (section 6.1), but also the Tasmanian samples all had quite high frequencies (about 0.25) of the *Mdh-1* 90 allele, an allele that was absent or only present at very low frequencies in all the overseas samples analysed. Either the Tasmanian animals were derived from a population with a low frequency of *Mdh-1* 90, or there exists an as yet unexamined overseas population with higher frequencies of *Mdh-1* 90, and which might more probably be the founder of the Tasmanian introductions. The first of these two possibilities could result either from the founder effect (the presumed few founding animals by chance having an atypically high frequency of the *Mdh-1* 90 allele) or from selection for this allele during transport or colonisation. The conclusions drawn from the genetic data essentially all rely on the assumption of genotype neutrality. If selection is shown to operate for particular genotypes in particular environments, then genetic data cannot be used to pinpoint possible sources of introductions, since genetically similar samples might reflect environmental similarities rather than genetic relatedness. However, there is no evidence for selection operating on the *Mdh-1* genotypes (nor on genotypes for any other locus). For example, the Tasmanian samples were in Hardy-Weinberg equilibrium for *Mdh-1*. Furthermore, environmental conditions are very different between the northern and southern Japanese samples, yet there were no differences in *Mdh-1*gene frequencies.

If it is assumed that the introductions were derived from ballast water dumping of Japanese woodchip boats visiting Triabunna, then these genetic data can be studied with respect to possible origins of wood chip boats. In the mid-late 1980s, three main ports sourced Japanese wood chip vessels (Bolch, pers. com.). These were Yatsushiro (near Kumamoto, on the Ariake sea on the southern island of Kyushu), Ishinomaki (on the main island of Honshu, near Sendai, about 350 km north of Tokyo Bay and about an equal distance south of Mutsu Bay), and Kushiro (about 100 km south of Nemuro Bay) (see Fig. 6.5). The genetic data indicate considerable differences between Ariake Sea samples and the Tasmanian animals. While no animals were collected from Kushiro, the Nemuro Bay sample quite close to this port was quite different from the Tasmanian samples. For example, it and all other northern samples analysed had a high frequency

of the 80 allele at the *Apk* locus (which shows far more population differentiation than any other locus), and it is likely that Kushiro animals would be similar. This allele was absent from the Tasmanian samples, which had the 100 allele as their dominant *Apk* allele, an allele at very low frequency in all northern Japanese samples. Therefore it seems unlikely that the introductions stemmed either from Yatsushiro or from Kushiro. This leaves Ishinomaki as the possible source. Interestingly, seven of eight Japanese vessels assayed for ballast water fauna at Triabunna by Williams *et al.* (1988) in 1976 and 1978 came from Ishinomaki. Unfortunately, no animals were examined from this region of Honshu, and it is not clear whether animals from this region would be expected to more closely match the northern samples or those from Tokyo Bay.

Of course, it is still possible that the introduction came from a Japanese port other than Ishinomaki, or indeed from a non-Japanese port, and may not be associated with woodchip boat movements at all. The genetic data indicate that it is unlikely that the source was the Ariake Sea (Kyushu), the north of Honshu, the island of Hokkaido, or the Russian port of Vladivostok: it is thus unlikely that the introduction was *A. amurensis versicolor* or a north Japanese strain. The introduction could have come from the Suruga Bay/ Tokyo Bay region of Honshu, from some other part of Honshu (other than the Mutsu Bay region in north Honshu), or from outside Japan. Additional investigations will be needed to further delimit its possible origin. If it is true that the source was central Honshu, then the animals could be adapted to quite warm summer water temperatures, and potentially could spread around southern Australian coasts. Sagara and Ino (1954) describe the upper lethal temperature for young *Asterias amurensis* from Tokyo Bay as 26^{o}C, and 20^{o}C for the bipinnaria larval stage.

- 2. Determine whether the Tasmanian populations are derived from one or several introductions.

The three Tasmanian samples analysed were genetically extremely similar to one another. No significant differences were found. There is therefore no evidence that the Triabunna population is derived from a separate introduction from the Derwent or D'Entrecasteaux Channel populations. These data are consistent with a single introduction to Tasmania, or (far less likely given the low probability of successful colonisation) multiple introductions from a single source population. The most plausible scenarios are either a single introduction to Triabunna followed by a spread to the Derwent and down the D'Entrecasteaux Channel, or an introduction to the Derwent river followed by a spread to Triabunna and down the D'Entrecasteaux Channel. For either scenario, the very high genetic similarity of Triabunna and Derwent populations suggests that the subsequent introduction, whether from Triabunna to the Derwent, or from the Derwent to Triabunna, represents the colonisation of an appreciable number of founders. Had only few founders been involved in this secondary colonisation, then it is likely that some small but significant genetic differences would have been detected between the original site of introduction and the secondary site.

- 3. Determine whether the invasion of Tasmanian waters has been accompanied by a loss of genetic variation with respect to native populations.

The average heterozygosity per locus is around 0.123 in the Tasmanian populations, and 0.177 to 0.216 (mean 0.192) in the native or overseas populations. Four loci that are monomorphic in the Tasmanian populations are polymorphic in all overseas

Fig. 6.5. Location of the six Japanese samples and the three major woodchip ports of Kushiro, Ishinomaki and Yatsushiro

populations studied. There is clear evidence that the introduction was accompanied by a loss of genetic variation, amounting to perhaps 33-36% of the original level.

Introductions do not inevitably lead to the loss of large amounts of variation. Table 6.9 summarises some observations made on seven species of molluscs, a group for which most data exists on the effects of introductions. All these introductions were accidental, rather than the deliberate transfer of, for example, commercial 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 6.9 that do not have planktonic larvae; both are ovoviviparous (laying egg masses from which miniature adults emerge).

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. Those 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. Indeed, the actual effect on average heterozygosity of a founding event *per se* may be rather small. Since each individual in a single panmictic population carries half the heterozygosity of that population, a single fertilised female carries 75% of the original population heterozygosity, and two fertilised females will carry 93.75%. Therefore while the initial loss of heterozygosity may be limited, heterozygosity will continue to be lost each generation at the rate of about 1/2N (where N is the effective population size): heterozygosity in small populations will be lost far faster than in large populations.

The loss of variation in introduced populations of *Asterias amurensis* appears to have been quite substantial, and perhaps too much to be simply attributed to a bottleneck caused by a limited numbers of founders. It is possible that population numbers remained very low for a few generations post-colonization, leading to the loss of additional variation. Variation has been lost at four loci polymorphic in all overseas populations (*Gpi, Pep-lp-2, Pep-vl-2, Hk*), gained at one locus (*Mdh-1)*, and maintained at four other loci (*Apk, Pep-vl-1, Pgdh, Pgm-2*).

While some variation has been lost in the process of colonisation, all samples, whether introduced or endemic, show quite high levels of variation (range 0.116 to 0.216, mean of 0.171). Mean heterozygosities per locus of 29 other species of seastars (Table 6.10) range from 0 (*Asterina minor*) to the extremely high figure of 0.33 (*Luidia quinaria*). The average heterozygosity per locus of the 36 studies listed in Table 6.10 (excluding our study but including other studies of *Asterias amurensis*) was 0.114. This is very similar to the average heterozygosity of 356 species of invertebrates, 0.122±0.004 (Ward *et al.*, 1992). It is abundantly clear that while the introduced Pacific seastars have lost some variation, they still retain a mean heterozygosity per locus of 0.123, and are not genetically depauperate relative to other seastars and other invertebrates.

OTHER OBSERVATIONS

Northern Pacific seastars from the Ariake Sea are considered sufficiently distinct, morphologically, to be classed as a separate subspecies, *Asterias amurensis versicolor.* On genetic grounds (Fig 6.1 and 6.2), if these animals are held to be a distinct subspecies, then the northern Japanese/Russian animals should likewise be considered a second subspecies, and the Tokyo Bay/Suruga Bay animals a third subspecies. Note, however, that all samples are quite closely related to one another, with overall Nei genetic identities in the range of 0.910-1.000, and distances in the range 0.095 to 0.000. While subspecies designations can be made, if desired, such values are consistent with the all samples being the same species: they do not suggest that, for example, *Asterias amurensis versicolor* should be considered a separate species.

Our results should also be briefly compared with other allozyme studies on this species. There are two published accounts of allozyme variation in this species (Table 6.10). One is by Manchenko (1986), of a single Russian population (probably the same one that we analysed from Vostok Bay), and one is by Matsuoka *et al.* (1994), of a single Japanese population from Mutsu Bay, almost certainly from the same area off Asamushi Marine Biological Station that our animals came from. A detailed comparison of allele frequencies is probably not worthwhile, given that the different investigators used different electrophoresis systems and different suites of loci (although a few loci were common to all studies). Both Manchenko's and Matsuoka *et al.*'s investigations revealed polymorphic loci, with average heterozygosities per locus of 0.202 and 0.076 respectively. Manchenko's average heterozygosity is similar to our value (0.213 for the Vladivostok population, 0.192 as a mean of the seven endemic populations), while Matsuoka *et al.*'s is considerably lower. Of the 29 loci examined by Matsuoka *et al.*, only seven were common to our study. However, these did include *Pgi* and *Hk*, which we found to be clearly polymorphic in Mutsu Bay but which Matsuoka *et al.* described as monomorphic. Thus the reduced average heterozygosity found by Matsuoka *et al.*. can be attributed not only to a different suite of loci, but also to electrophoresis systems which failed to resolve some variation.

The high degree of genetic differentiation among the endemic Japanese and Russian populations deserves some comment. Marine invertebrates with planktonic larvae typically show low levels of population differentiation. This is presumably largely a consequence of gene flow brought about by passive egg and larval dispersal in ocean currents: invertebrates which are direct developers show much more population differentiation (Ward, 1990; Hunt, 1993). Table 6.11 summarises information about the extent of differentiation among populations of seastars. These are consistent with the assumption that planktonic larval drift enhances gene flow. With the exception of *Asterias amurensis,* F_{ST} values (F_{ST} and G_{ST} measure the same quantity, the amount of the total genetic variation attributable to population differentiation) for those species with planktonic larvae and thus high dispersal abilities are all low, ranging from 0-0.072. On the other hand, those species lacking planktonic larvae all have high F_{ST} values, ranging from 0.332-0.471.

Table 6.9. Losses of genetic variation consequent upon accidental introductions of 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	NO. OF LOCI	AVERAGE HETEROZYGOSITY ENDEMIC	INTROD.	% CHANGE	REFERENCE
Asterias amurensis (Japan/Russia vs. Tasmania)	22	0.192^{7} 0.185^{2*}	0.123^{3} 0.123^{3}	-35.9 -33.5	present study
(a) 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.
(b) Freshwater mollusc					
Dreissena polymorpha (Europe vs. Great Lakes)	11	0.451^{3}	0.465^{7}	+3.1	Boileau and Hebert, 1993.
(c) Land mollusc					
Theba pisana (France vs. Australia)	25	0.176^{2**}	0.086^{2}	-51.1	Johnson, 1988.

*includes only Tokyo Bay and Suruga Bay, judged more likely than the others to be source populations

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

Table 6.10. Summary of genetic variation in seastars of the class Asteroidea

SPECIES	AVERAGE HETEROZYGOSITY*	NO. OF LOCI	REFERENCE
ORDER FORCIPULATA			
Asterias amurensis	0.171	22	present study
Asterias amurensis	0.076	29	Matsuoka *et al.*, 1994
Asterias amurensis	0.202	36	Manchenko, 1986
Asterias forbesi	0.041	27	Schopf and Murphy, 1973
Asterias vulgaris	0.023	27	Schopf and Murphy, 1973
Coscinasterias acutispina	0.061	30	Matsuoka *et al.*, 1991
Coscinasterias acutispina	0.085	28	Matsuoka *et al.*, 1994
Aphelasterias japonica	0.090	15**	Matsuoka, 1991
Aphelasterias japonica	0.064	28	Matsuoka *et al.*, 1994
Aphelasterias japonica	0.173	24	Manchenko, 1986
Distolasterias nippon	0.166	26	Matsuoka *et al.*, 1993
Distolasterias nippon	0.086	34	Manchenko, 1986
Plazaster borealis	0.059	26	Matsuoka *et al.*, 1994
Myxoderma sacculatum ectenes	0.144	14	Ayala *et al.*, 1975
Leptasterias epichlora	0.164	14	Kwast *et al.*, 1990
Leptasterias epichlora	0.135	16	Stickle *et al.*, 1992
Leptasterias hexactis	0.254	14	Kwast *et al.*, 1990
Leptasterias hexactis	0.151	16	Stickle *et al.*, 1992
Leptasterias polaris	0.083	25	Stickle *et al.*, 1992
Evasterias troschelii	0.050	25	Stickle *et al.*, 1992
Evasterias retifera	0.072	23	Manchenko, 1986
Lethasterias fusca	0.142	27	Manchenko, 1986
Lysastrosoma anthosticta	0.076	30	Manchenko, 1986
Pisaster ochraceus	0.092	24	Stickle *et al.*, 1992
ORDER SPINULOSA			
Acanthaster planci	0.200	14	Nishida and Lucas, 1988
Acanthaster brevispinus	0.155	14	Nishida and Lucas, 1988
Asterina pectinifera	0.029	14	Matsuoka, 1981
Asterina batheri	0.087	16	Matsuoka, 1981
Asterina coronata japonica	0.033	15	Matsuoka, 1981
Asterina pseudoexigua pacifica	0.038	13	Matsuoka, 1981
Asterina minor	0	14	Matsuoka, 1981
Pteraster jordani	0.101	24	Ayala *et al.*, 1975
Diplopteraster multipes	0.108	18	Ayala *et al.*, 1975
Patiria pectinifera	0.174	23	Manchenko, 1986
Henricia leviuscula	0.210	30	Manchenko, 1986
ORDER PHANEROZONIA			
Nearchaster aciculosus	0.195	24	Ayala *et al.*, 1975
Luidia quinaria	0.327	25	Manchenko, 1986

*Hardy-Weinberg expected values; ** Only abstract of paper available, which refers to 15 enzymes. This probably represents more than 15 loci.

It is clear that *Asterias amurensis* (with an F_{ST} of 0.096 for the endemic populations) shows a relatively high level of genetic population differentiation for a seastar with planktonic larvae. While the length of the planktonic stage under natural conditions remains uncertain, it is likely to be at least six weeks and possibly substantially longer. Sagara and Ino (1954) cultured the bipinnaria (pre-settlement) larval stage over a 40 day period, Bruce (pers.com.) reared larvae for 7 weeks to the bipinnaria stage, Kasyanov (unpublished report) reared larvae for 7-8 weeks to the brachiolaria stage, and Komatsu (pers. com.) maintained larvae for 5 months without settlement. Given the extended duration of the larval phase, and consequent high vagility of the species, how can the high levels of population differentiation in this species be accounted for?

Population differentiation can, in principle, arise from genetic drift, selection, or a combination of these two forces. The samples of *Asterias amurensis* from Japan came from quite different environments: the northern populations from Hokkaido come from considerably cooler waters than the southern Japanese sample from Kyushu. This environmental range is greater than for any other species listed in Table 6.11. While some of the *Acanthaster planci* (Crown-of-thorns starfish) samples come from geographically more distant localities, this species is restricted to tropical seas. It is possible that in *Asterias amurensis,* local selective forces, varying among localities, override the effects of gene flow. However, for the most differentiated locus, *Apk*, the southern sample is genetically intermediate between the mid-Japan and northern Japan samples (Figs 6.3 and 6.4). This is not what would be expected on any simple selection model based on clinally varying selective factors. Such models would have predicted the northern and southern samples to show the greatest differences. It is possible that while the *Apk* polymorphism itself may not be under selection, *Apk* alleles may be in linkage disequilibrium with an unidentified polymorphism that is under selection. However, any such linkage disequilibrium would be expected gradually to diminish and eventually disappear unless the linkage is very tight. We have no evidence for selection operating on any of the allozyme polymorphisms described in this seastar.

Possibly the three endemic groups of *Asterias amurensis* identified in this study are derived from three populations that were once spatially and reproductively isolated, perhaps by sea-level changes brought about by Pleistocene glaciations, and insufficient time has elapsed for the gene pool to have become homogeneous. Whatever the source of the inter-population differentiation, its existence is most striking and affords opportunities for delimiting still more closely the likely origin of the Tasmanian animals.

Table 6.11 Estimates of levels of population differentiation (F_{ST} or G_{ST}) for seastars

SPECIES AND SCALE OF STUDY	EXTENT OF POPULATION DIFFERENTIATION (F_{ST} OR G_{ST}) RANGE/LOCUS	MEAN	REFERENCE
(A) Seastars with high dispersal abilities (planktonic larvae)			
Asterias amurensis			
Japan, Vladivostok (1,750 km)	0-0.468	0.096	present study
Acanthaster planci			
Pacific (12,500 km)	0.005-0.149	0.072	Nishida and Lucas, 1988
W. Pacific (2,500 km)	0-0.090	0.023	Nishida and Lucas, 1988
W & E. Australia (6,300 km)	0.008-0.075	0.030	Benzie and Stoddart, 1992
Linckia laevigata			
Great Barrier Reef (>1000 km)	0-0.005	0.001	Williams and Benzie, 1993
Patiriella calcar			
S.E. Australia (230 km)	0-0.003	0.000	Hunt, 1993
Evasterias troschelii			
Alaska (250 km)	0-0.053**	0.013**	Stickle *et al.*, 1992
Pisaster ochraceus			
Alaska (650 km)	0-0.029**	0.007**	Stickle *et al.*, 1992
(B) Seastars with low dispersal abilities			
Leptasterias epichlora[a]			
NW. USA (1500km)	0.001-0.614**	0.471*	Kwast *et al.*, 1990
Leptasterias hexactis[a]			
NW. USA (1500 km)	0.005-0.609**	0.332*	Kwast *et al.*, 1990
Coscinasterias calamaria[b]			
W. Australia (40 km)	-	0.358*	Johnson & Threlfall, 1987
Patiriella exigua[c]			
S.E. Australia (230 km)	0.080-0.601	0.462	Hunt, 1993

*Calculated by Williams and Benzie, 1993.
** Calculated from data supplied in original paper

[a]Females brood eggs and embryos
[b]Asexual reproduction (fissiparity) predominates over sexual recruitment
[c]Juveniles develop directly from egg masses deposited directly on rocky shores

7. BENEFITS

The introduction of *Asterias* to Tasmanian waters is considered to be a problem of national significance. The potential impact on both the fishing industry (primarily shellfish fisheries and aquaculture) and marine ecosystems is considerable. Thus beneficiaries of this research extend to all major southern Australian shellfish fisheries, aquaculture operations and fin fish fisheries via the marine ecosystem. This work has shown that the introduction is unlikely to be of the southern Japanese subspecies, *Asterias amurensis versicolor*, and therefore is unlikely to spread as far north as that subspecies might. It has also shown that although some genetic variation has been lost during colonisation, the introduced populations still exhibit considerable genetic diversity, possibly retarding attempts at biological control as it may be easier to control a genetically depauperate population.

8. INTELLECTUAL PROPERTY

No commercial intellectual property arose from this work.

9. FURTHER DEVELOPMENT

This project should be developed in two ways: one, to provide a still more powerful analysis of population relationships, and two, to enable unambiguous genetic larval identification. Furthermore, should there be any outbreaks on the mainland of Australia, these should be genetically examined to determine their likely source as Tasmania or overseas.

9.1. FURTHER POPULATION CHARACTERISATION

In order to provide a more powerful study of the possible source of the Tasmanian introduction than was possible in this preliminary project, it is necessary to do two things:

1. Examine further populations from the North Pacific Ocean. It would be especially valuable to examine specimens from the area of Ishinomaki/Sendai (Honshu), and from southern Honshu. Attempts were made during this project to collect a sample from the Seto Inland Sea, but apparently the species is extremely rare there (I. Imai, pers.com). It would also be interesting to examine one or more collections from the western coast of Honshu. Samples from areas other than Japan and Vladivostok should also be examined. It would be especially interesting to examine animals from Korea and Alaska, thus greatly extending coverage of the endemic range of this species.

2. The allozyme analysis should be supplemented with a DNA analysis. This will increase the power of the analyses considerably by providing additional genetic markers. In particular, mitochondrial DNA (mtDNA) should be analysed, since mtDNA is more sensitive to genetic drift than nuclear DNA (which encodes allozymes) and evolves faster. Although DNA analysis always takes longer than allozyme analysis, it should be possible to develop mtDNA methodologies quite rapidly. An appropriate mtDNA approach would be to amplify up sections of mtDNA using the polymerase chain reaction, and then examine these amplified regions either by DNA sequence analysis or

by 4 cutter restriction enzyme digestion. This would also make good use of the valuable resource we now have in our freezers of Japanese and Russian samples.

Note that the more characters that can be used to describe samples, the more powerful becomes any analysis of population relationships. So not only will it be possible to compare the introduced animals more rigorously with overseas samples, it will increase the power of tests aimed at determining whether any outbreaks on the mainland are more likely to have come from Tasmanian or northern Pacific localities.

9.2. LARVAL IDENTIFICATION

The advantages of developing mtDNA analyses of *Asterias amurensis* do not solely relate to determining population relationships: they will also enable unequivocal species identification of any stage. The correct identification of starfish larvae is crucial to the success of research projects which aim to use larval information to develop models of dispersal. There are several species of starfish in southern Tasmanian waters with planktonic larvae, but there are currently no morphological keys to their larval identification. The larvae of three species of starfish are now being reared and described, but at this stage it appears that plasticity in shape means that morphological identification to species level will be difficult. While variation in spawning season gives additional clues to larval identifications, at this stage it appears likely that, of the methods available, only genetic characterisation will provide unambiguous identification. It should also be pointed out that this will also permit the identification of *Asterias amurensis* larvae found in ballast water. This genetic characterisation will be based on PCR amplification and analysis of known regions of the mtDNA genome. This technique not only requires much less initial material than allozyme analyses, but permits the testing of alcohol-stored as well as fresh or frozen material.

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STAFF

Bob Ward	CSOF7	Principal Investigator
Jane Andrew	CSOF3	Research Assistant

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11. REFERENCES

Ayala, F.J., Valentine, J.W., Hedgecock, D. and Barr, L.G. 1975. Deep-sea asteroids: high genetic variability in a stable environment. *Evolution* 29, 203-212.

Benzie, J.A.H. and Stoddart, J.A. 1992. Genetic structure of crown-of-thorns starfish (*Acanthaster planci*) in Australia. *Marine Biology* 112, 631-639.

Boileau, M.G. and Hebert, P.D.N. 1993. Genetics of the zebra mussel (*Dreissena polymorpha*) in populations from the Great Lakes region and Europe. In, *Zebra mussels: biology, impacts and control*. Eds Nalepa, T.F. and Schloesser, D. Lewis Publishers, Ann Arbor, MI., USA.

Carlton, J.T. and Geller, J.B. 1993. Ecological roulette: the global transport of nonindigenous marine organisms. *Science* 261, 78-82.

Chakraborty, R. and Nei, M. 1977. Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. *Evolution* 31, 347-356.

Davenport, S. and McLoughlin, R. 1993. Preliminary assessment of the distribution and potential impact of the introduced starfish *Asterias amurensis* in Tasmanian waters. *Status Report to Fisheries Research and Development Corporation. June 1993, CSIRO.*

D'Yakanov, A.M. 1968. *Sea Stars (Asteroids) of the USSR Seas*. Israel Program for Scientific Translations, Jerusalem.

Elliott, N.G. and Ward, R.D. 1992. Enzyme variation in orange roughy, *Hoplostethus atlanticus* (Teleostei: Trachichthyidae), from southern Australian and New Zealand waters. *Australian Journal of Marine and Freshwater Research* 43, 1561-1571.

Galtsoff, P.S. and Loosanoff, V.L. 1939. Natural history and method of controlling the starfish (*Asterias forbesi,* Desor). *Fishery Bulletin* 49, 74-132.

Grant, W.S. and Cherry, M.I. 1985. *Mytilus galloprovincialis* Lmk. in southern Africa. *Journal of Experimental Marine Biology and Ecology* 90, 179-191

Hebert, P.D.N. and Beaton, M.J. 1989. *Methodologies for allozyme analysis using cellulose acetate electrophoresis*. Helena Laboratories, Beaumont, Texas.

Hebert, P.D.N., Muncaster, B.W. and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 46, 1587-1591.

Hoagland, K.E. 1985. Genetic relationships between one British and several North American populations of *Crepidula fornicata* based on allozyme studies. *Journal of Molluscan Studies* 51, 177-182.

Hunt, A. 1993. Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of two intertidal starfish, *Patiriella calcar* and *P. exigua*. *Marine Ecology Progress Series* 92, 179-186.

Johnson, D. 1994. Seastar fight gains momentum. *Australian Fisheries* 53 (1), 25-27.

Johnson, M.S. 1988. Founder effects and geographic variation in the land snail *Theba pisana*. *Heredity* 61, 133-142.

Johnson, M.S. and Threlfall, T.J. 1987. Fissiparity and population genetics of *Coscinasterias calamaria*. *Marine Biology* 93, 517-525.

Kasyanov, V.L. 1988. Reproductive strategies of seastars in the Sea of Japan. In, *Echinoderm biology,* 1st ed. Eds. Burke, R.D., Mladenov, P.V., Lambert, P. and Parsley, R.L. A.A. Balkema, Rotterdam.

Knight, A.J., Hughes, R.N. and Ward, R.D. 1987. A striking example of the founder effect in the mollusc *Littorina saxatilis*. *Biological Journal of the Linnean Society,* 32, 417-426.

Kwast, K.E., Foltz, D.W. and Stickle, W.B. 1990. Population genetics and systematics of the *Leptasterias hexactis* (Echinodermata: Asteroidea) species complex. *Marine Biology* 105, 477-489.

Manchenko, G.P. 1986. Electrophoretic estimation of the level of intraspecific genetic variability in seastars from the Sea of Japan. *Marine Biology, Vladivostok,* 6, 43-52 (in Russian).

Matsuoka, N. 1981. Phylogenetic relationships among five species of starfish of the genus *Asterina*: an electrophoretic study. *Comparative Biochemistry and Physiology* 70B, 739-743.

Matsuoka, N. 1991. Maintenance mechanism of enzyme polymorphism in echinoderms. *Science Reports of Hirosaki University* 38, 38-45.

Matsuoka, N., Yoshida, K., Fukuda, K. and Shigei, M. 1991. Genetic variation in the starfish *Coscinasterias acutispina*. *Comparative Biochemistry and Physiology* 99B, 893-898.

Matsuoka, N., Inamori, M. and Sugawara, M. 1993. High genetic variability in the starfish *Distolasterias nippon* revealed by enzyme electrophoresis. *Comparative Biochemistry and Physiology* 104B, 75-79.

Matsuoka, N., Fukuda, K., Yoshida, K., Sugawara, M. and Inamori, M. 1994. Biochemical systematics of five asteroids of the family Asteriidae based on allozyme variation. *Zoological Science* 11, 343-349.

Meehan, B.W., Carlton, J.T. and Wenne, R. 1989. Genetic affinities of the bivalve *Macoma balthica* from the Pacific coast of North America: evidence for recent introduction and historical distribution. *Marine Biology* 102, 235-241.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science, USA* 70, 3321-3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583-590.

Nei, M., Maruyama, T. and Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. *Evolution* 29, 1-10.

Nishida, M. and Lucas, J.S. 1988. Genetic differences between geographic populations of the Crown-of-thorns starfish throughout the Pacific region. *Marine Biology* 98, 359-368.

Nojima, S., Soliman, F. E., Kondo, Y., Kuwano, Y., Nasu, K. and Kitajima, C. 1986. Some notes on the outbreak of the sea star, *Asterias amurensis versicolor* Sladen, in the Ariake Sea western Kyushu. *Publications from the Amakusa Marine Biological Laboratory, Kyushu University*, 8, 89-112.

Onguru, C. and Okutani , T., eds. 1991. *Echinoderms from Continental Shelf and Slope around Japan,* 1st ed., Vol. II, Japan Fish. Res. Conservation Association, Tokyo, 204pp.

Roff , D.A., and Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: c2 and the problem of small samples. *Molecular Biology and Evolution* 6, 539-545.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. *Studies in Genetics* VII, University of Texas Publications 7213, 145–153.

Sagara, J.I. and Ino, T. 1954. The optimum temperature and specific gravity for bipinnaria and young of Japanese starfish, *Asterias amurensis* Lutken. *Bulletin of the Japanese Society of Scientific Fisheries* 20, 689-693.

Schopf, T.J.M. and Murphy, L.S. 1973. Protein polymorphism of the hybridizing sea-stars *Asterias forbesi* and *Asterias vulgaris* and implications for their evolution. *Biological Bulletin of the Marine Biology Laboratory, Woods Hole.* 145, 589-597.

Stickle, W.B., Foltz, D.W., Katoh, M. and Nguyen, H.L. 1992. Genetic structure and mode of reproduction in five species of sea stars (Echinodermata: Asteroidea) from the Alaskan coast. *Canadian Journal of Zoology* 70, 1723-1728.

Turner, E. 1992. A northern Pacific seastar, *Asterias amurensis,* in Tasmania. *Australian Marine Science Association, Bulletin* 120, 18-19.

Ward, R.D. 1990. Biochemical genetic variation in the genus *Littorina. Hydrobiologia*,193, 53-69.

Ward, R.D., Skibinski, D.O.F. and Woodwark, M. 1992. Protein heterozygosity, protein structure, and taxonomic differentiation. *Evolutionary Biology* 26, 73-159.

Williams, R.J., Griffiths, F.B., van der Wal, E.J. and Kelly, J. 1988. Cargo vessel ballast water as a vector for the transport of non-indigenous marine species. *Estuarine, Coastal and Shelf Science* 26, 409-420.

Williams, S.T. and Benzie, J.A.H. 1993. Genetic consequences of long larval life in the starfish *Linckia laevigata* (Echinodermata: Asteroidea) on the Great Barrier Reef. *Marine Biology* 117, 71-77.

Woodruff, D.S., McMeekin, L.L., Mulvey, M. and Carpenter, M.P. 1986. Population genetics of *Crepidula onyx*: variation in a Californian slipper snail recently established in China. *Veliger* 29, 53-63.