

INDICES OF GENETIC IDENTITY AND DISTANCE IN THREE TAXA
WITHIN THE *BALANUS AMPHITRITE* DARWIN COMPLEX
(CIRRIPEDIA, THORACICA)

BY

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INTRODUCTION

Balanus amphitrite Darwin, 1854, is an extremely variable species with a world-wide distribution in warm temperate and tropical waters. Darwin originally described nine varieties of the species *amphitrite*. Because of his incomplete description of the localities and type specimens and incomplete diagnosis, much confusion has ensued in the literature, as subsequent investigators have tried to relate taxa making up the *B. amphitrite* complex to the varieties originally described by Darwin. A resumé of the literature and a summary of the taxonomic confusions within the *amphitrite* complex have been outlined by Henry & McLaughlin (1975). Despite the redescription and revision of Darwin's type specimens by Harding (1962), sufficient anomalies remained for Henry & McLaughlin (1975) to undertake a revision of all described taxa in the *amphitrite* complex. Using both classical and statistical approaches, they have divided the complex into 23 taxa, forming 5 subgroups.

In recent years, electrophoresis of soluble proteins has been used to help elucidate problems of variation, speciation and taxonomy within the balanomorph cirripedes. Nevo et al. (1977, 1978), studied the effects of thermal selection and pollution on populations of *Balanus amphitrite* Darwin, 1854. Three partially sympatric sibling species of the *Chthamalus fissus* Darwin, 1854, group have been indicated by Hedgecock (1979) with genetic distances between them at least as large as those between *C. fissus* and the distinct species *C. dalli* Pilsbry, 1916 and *C. anisopoma* Pilsbry, 1916. *Chthamalus* species in western Europe have been investigated (Juan, 1976; Dando et al., 1979). Races of *Balanus balanoides* (L.) (cf. Darwin, 1854) have been described (Flowerdew & Crisp, 1975; Flowerdew, 1983a, b) and genetic comparisons have been made between immigrant and aboriginal populations of *Elminius modestus* Darwin, 1854 (cf. Flowerdew, 1984).

With a view to elucidating further some of the interrelationships within the *Balanus amphitrite* complex, electrophoresis was carried out on three taxa within

the complex, namely *B. amphitrite amphitrite* Darwin, 1854 (originally described as *B. amphitrite* var. *communis* by Darwin, 1854), *Balanus variegatus* Darwin, 1854 (originally described by Darwin as *Balanus amphitrite variegatus*) and *Balanus amphitrite saltonensis* Rogers, 1949.

MATERIALS AND METHODS

Samples were collected over a two year period and electrophoresis was carried out using fresh animals as they became available. Samples (with dates) were taken from Oakland, California (March, 1981); Kane Spring, Salton Sea, California (April, 1982); Mission Bay, San Diego, California (August, 1982); Bombay (December, 1982); Wu Kai Sha Jetty, Tolo Harbour, Hong Kong (April, 1983) and East Coast Parkway, Singapore (June, 1983). *Balanus variegatus* was collected from Changi, Singapore (June, 1983). The samples were prepared by removing the animal from the substratum and pushing the tergum and scutum with attached musculature and prosoma through the base of the animal. The tissues were homogenised in iced, de-ionised, distilled water (circa 1:4, w:v) and kept at 4°C for 2 h prior to centrifugation at 10,000 *g* for 3 min. The supernatant was absorbed on to 10 × 5 mm pieces of Whatman No. 3 chromatography paper and inserted into the origin of previously prepared 12% starch gels (Connaught Laboratories). The enzymes systems examined and running conditions are given in table I. Staining solutions were prepared generally following the recipes given by Harris & Hopkinson (1976) with 50 µl 0.8% Meldola's Blue substituted for phenazine methosulphate except in the preparation used to visualize superoxide dismutase. Horse spleen ferritin (Sigma Chemical Co.) was included as a marker protein on each gel.

Allozymes have been designated by their mobility (RM) relative to the most frequent band at each locus, designated by the superscript 100. When an enzyme was found to be encoded at more than one locus, the lower number indicates the more anodal locus.

RESULTS AND DISCUSSION

The allele proportions and heterozygosity values, at a maximum of 18 loci for each sample, are presented in tables II and III. Indices of genetic identity *I* and genetic distance *D* (Nei, 1972) comparing *Balanus amphitrite amphitrite* and *B. amphitrite saltonensis* are contained in table III. The values of *I* and *D* based on 31 alleles at 11 loci were found to be in the range of variation expected between conspecific populations (Avisé, 1974; Thorpe, 1979). Since the indices are more severely affected by the number of loci studied than by small sample size (Nei, 1978; Gorman & Renzi, 1979), the small number of individuals in the Mission Bay sample should not significantly influence the result.

The values of *I* and *D* based on 64 alleles at 17 loci comparing *Balanus amphitrite amphitrite* and *B. variegatus* are given in table V. The range of genetic

TABLE I

Electrophoresis of *Balanus amphitrite*, enzymes examined, running conditions and buffers used

Enzyme	E.C. number	Buffers used
L-Iditol dehydrogenase (SORDH)	1.1.1.14	B
malate dehydrogenase (MDH)	1.1.1.37	A
malic enzyme (ME)	1.1.1.40	B
isocitrate dehydrogenase (ICD)	1.1.1.42	A
phosphogluconate dehydrogenase (PGD) (decarboxylating)	1.1.1.44	A
superoxidase dismutase (SOD)	1.15.1.1	A
phosphoglycerate kinase (PGK)	2.7.3.2	A
phosphoglucomutase (PGM)	2.7.5.1	A
β -N-acetylglucosaminidase (HEX)	3.2.1.30	A
β -glucuronidase (β -GUS)	3.2.1.31	A
lumarate hydratase (FH)	4.2.1.2	A
mannose phosphate isomerase (MPI)	5.3.1.8	A
glucose phosphate isomerase (GPI)	5.3.1.9	A

A: electrode buffer: 0.1 M Tris, 0.1 M maleic acid, 0.01 M EDTA (Na_2), 0.01 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ to pH 7.4 with 40% NaOH solution; gel buffer 1:9 dilution of electrode buffer run at 90V for 17 h at 4°C.

B: electrode buffer: 0.3 M boric acid adjusted to pH 8.6 with 40% NaOH solution gel buffer: 0.014 M Tris to pH 8.6 with citric acid run at 250V for 3 h at 4°C.

identity values between *B. a. amphitrite* and *B. variegatus* was found to be 0.392-0.399, typical values for species within a genus where values of *I* range from 0.25-0.85 (Thorpe, 1979).

Balanus amphitrite saltonensis is known only from the land-locked Salton Sea in southern California and an anomalous sample taken from Wilmington Harbour, Los Angeles in 1914 (Henry & McLaughlin, 1975). The subspecies was first described by Rogers (1949). He also described two other subspecies of *B. amphitrite* in San Francisco Bay. But these have now been ascribed to the subspecies *B. a. amphitrite* by Henry & McLaughlin (1975), who have maintained *B. a. saltonensis* as a subspecies but stressed its very close alliance to *B. a. amphitrite*. Nei's genetic identity index based on 11 loci described herein, however, shows no significant genetic differentiation of *B. a. saltonensis* from *B. a. amphitrite* populations sampled over a wide geographical range. This result suggests that *B. a. saltonensis* should be reconsidered as *B. a. amphitrite*.

Balanus variegatus was first described by Darwin (1854) as a variety of *B. amphitrite*. Harding (1962) assigned the variety to its own species *B. variegatus*, a position maintained by Henry & McLaughlin (1975) in their major redescription of the *amphitrite* complex. The present study fully confirms the species status for *B. variegatus*.

TABLE II

Allele proportions at 18 loci in *Balanus amphitrite amphitrite* and *Balanus variegatus*.*N* = no. of individuals examined; n.e. = not examined

Locus	RM	<i>B. amphitrite saltonensis</i>		Allele proportions <i>Balanus amphitrite amphitrite</i>								<i>B. variegatus</i>				
		Salton Sea California	<i>N</i>	Oakland California	<i>N</i>	Mission Bay	<i>N</i>	Bombay	<i>N</i>	Hong Kong	<i>N</i>	Singapore	<i>N</i>	Singapore	<i>N</i>	
<i>MDH-1</i>	110	n.e.		0	38	0	10	0	70	0	50	0.010	48	0.011	45	
	100			1.000		1.000		1.000		1.000		0.969		0.989		
	90			0		0		0		0		0.021		0		
<i>MDH-2</i>	100	n.e.		1.000	38	1.000	10	1.000	70	1.000	50	1.000	48	1.000	45	
<i>SORDH</i>	100	1.000	45	1.000	38	1.000	15	1.000	50	1.000	50	1.000	36	1.000	36	
<i>ME</i>	100	1.000	45	1.000	38	1.000	15	1.000	50	1.000	50	1.000	36	1.000	36	
<i>ICD-1</i>	142	0	112	0	28	0	27	0	90	0	86	0	126	0.019	54	
	131	0		0		0		0		0		0		0.009		
	123	0		0		0		0.006		0.006		0		0.963		
	115	0.473		0.464		0.407		0.022		0.366		0.056		0.009		
	110	0		0.036		0		0		0		0		0		
	100	0.509		0.500		0.555		0.933		0.611		0.926		0		
	86	0.018		0		0.019		0.033		0.017		0.019		0		
	73	0		0		0.019		0.006		0		0		0		
	<i>ICD-2</i>	127	0	112	0	28	0	27	0	90	0	86	0	126	0.009	54
		117	0		0		0.019		0		0.035		0.009		0.982	
100		1.000		1.000		0.981		1.000		0.959		0.991		0.009		
76		0		0		0		0		0.006		0		0		
<i>AGD</i>	111	0.009	117	0	37	0	14	0	40	0	80	0	55	0	58	
	100	0.961		1.000		1.000		0.987		0.969		0.982		0.009		
	90	0.030		0		0		0		0		0		0.991		
	85	0		0		0		0.013		0.025		0.009		0		
	70	0		0		0		0		0.006		0.009		0		

	76	0		0		0		0		0		0		0	
<i>PGD</i>	111	0.009	117	0	37	0	14	0	40	0	80	0	55	0	58
	100	0.961		1.000		1.000		0.987		0.969		0.982		0.009	
	90	0.030		0		0		0		0		0		0.991	
	85	0		0		0		0.013		0.025		0.009		0	
	70	0		0		0		0		0.006		0.009		0	

TABLE II (cont'd)

Locus	RM	Allele proportions													
		<i>B. amphitrite saltonensis</i>			<i>Balanus amphitrite amphitrite</i>						<i>B. variegatus</i>				
		Salton Sea California	N	Oakland California	N	Mission Bay	N	Bombay	N	Hong Kong	N	Singapore	N	Singapore	N
<i>SOD</i>	100	1.000	50	1.000	38	1.000	14	0.931	29	1.000	83	1.000	20	0.025	20
	90	0		0		0		0		0		0		0.100	
	77	0		0		0		0.069		0		0		0.875	
<i>PGK</i>	115	n.e.		n.e.		n.e.		0.013	86	0.006	88	0.011	47	0.022	46
	100							0.987		0.994		0.958		0.957	
	85							0		0		0.032		0.022	
<i>PGM-1</i>	120	0	118	0	37	0	29	0	78	0	78	0	57	1.000	56
	100	1.000		1.000		1.000		1.000		1.000		1.000		0	
<i>PGM-2</i>	141	0	118	0	37	0	29	0	78	0	78	0	57	0.027	56
	126	0		0		0		0		0		0		0.929	
	117	0.004		0		0		0.006		0		0		0.018	
	110	0.051		0.081		0.102		0.013		0.096		0.044		0.018	
	100	0.894		0.824		0.898		0.897		0.808		0.904		0	
	95	0		0		0		0		0		0		0.009	
	88	0.042		0.095		0		0.045		0.071		0.035		0	
	77	0.009		0		0		0.039		0.026		0.018		0	
<i>PGM-3</i>	100	1.000	118	1.000	37	1.000	29	1.000	78	1.000	78	1.000	57	1.000	56
<i>HEX-1</i>	125	n.e.		0	10	0	21	0	88	0	85	0	39	1.000	44
	109			0		0.024		0.011		0.006		0		0	
	100			1.000		0.952		0.977		0.971		0.987		0	
	90			0		0.024		0.011		0.024		0.013		0	
<i>HEX-2</i>	147	n.e.		0	10	0	21	0	88	0	85	0	39	0.978	44
	132			0		0		0		0		0		0.022	
	115			0.050		0.024		0.114		0.088		0		0	
	100			0.950		0.976		0.886		0.912		1.000		0	

TABLE II (cont'd)

Locus	RM	Allele proportions													
		<i>B. amphitrite saltonensis</i>				<i>Balanus amphitrite amphitrite</i>				<i>B. variegatus</i>					
		Salton Sea California	<i>N</i>	Oakland California	<i>N</i>	Mission Bay	<i>N</i>	Bombay	<i>N</i>	Hong Kong	<i>N</i>	Singapore	<i>N</i>	Singapore	<i>N</i>
<i>β-GUS</i>	110	n.e.		0	10	0	21	0	70	0	50	0	47	1.000	53
	100			1.000		1.000		0.986		1.000		0.977		0	
	90			0		0		0		0		0.023		0	
	87			0		0		0.014		0		0		0	
<i>FH</i>	100	1.000	50	1.000	10	1.000	20	1.000	70	1.000	50	1.000	47	0	45
	88	0		0		0		0		0		0		1.000	
<i>MPI</i>	118	0.010	102	0.028	18	0.019	26	0.035	85	0.012	85	0.015	34	0.021	47
	110	0.427		0.472		0.308		0.341		0.329		0.177		0	
	100	0.520		0.472		0.635		0.506		0.547		0.618		0.096	
	96	0		0		0		0		0		0		0.160	
	90	0.044		0.028		0.019		0.077		0.106		0.162		0	
	83	0		0		0.019		0.029		0.006		0.029		0.543	
	77	0		0		0		0		0		0		0.117	
	67	0		0		0		0.012		0		0		0.053	
	60	0		0		0		0		0		0		0.011	
<i>GPI</i>	175	0	118	0	38	0	29	0.044	90	0	78	0.006	57	0.025	80
	169	0		0		0		0		0		0		0.125	
	160	0.047		0.079		0		0.039		0.039		0		0.019	
	138	0.064		0		0		0.178		0.122		0.063		0	
	127	0.004		0.013		0.017		0.006		0		0.044		0.806	
	100	0.855		0.856		0.948		0.694		0.782		0.815		0	
	96	0		0		0		0		0		0		0.025	
	64	0.013		0.023		0.035		0.017		0.051		0.060		0	
	41	0.017		0.030		0		0.022		0.006		0.006		0	
	35	0		0		0		0		0		0.006		0	

	0.000	0.000	0.040	0.094	0.782	0.815	0
96	0	0	0	0	0	0	0.025
64	0.013	0.023	0.035	0.017	0.051	0.060	0
41	0.017	0.030	0	0.022	0.006	0.006	0
35	0	0	0	0	0	0.006	0

TABLE III

Observed and expected heterozygosity values at 12 polymorphic loci in *Balanus amphitrite amphitrite* and *Balanus variegatus*. H_o = observed heterozygosity; H_e = expected heterozygosity; n.e. = not examined

Locus	<i>B. a. saltonensis</i>		<i>Balanus amphitrite amphitrite</i>								<i>B. variegatus</i>			
	Salton Sea		Oakland		Mission Bay		Bombay		Hong Kong		Singapore		Singapore	
	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e
<i>MDH-1</i>	n.e.		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.061	0.022	0.022
<i>ICD-1</i>	0.429	0.517	0.482	0.533	0.482	0.514	0.133	0.128	0.500	0.492	0.130	0.139	0.074	0.072
<i>ICD-2</i>	0.000	0.000	0.000	0.000	0.074	0.037	0.000	0.000	0.029	0.059	0.019	0.018	0.037	0.036
<i>PGD</i>	0.078	0.092	0.000	0.000	0.000	0.000	0.025	0.026	0.063	0.061	0.036	0.036	0.017	0.018
<i>SOD</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.137	0.128	0.000	0.000	0.000	0.000	0.200	0.224
<i>PGK</i>	n.e.		n.e.		n.e.		0.024	0.026	0.011	0.012	0.085	0.081	0.087	0.083
<i>PGM-2</i>	0.195	0.196	0.324	0.305	0.138	0.183	0.167	0.192	0.372	0.332	0.192	0.179	0.143	0.136
<i>HEX-1</i>	n.e.		0.000	0.000	0.133	0.093	0.034	0.044	0.035	0.057	0.026	0.026	0.000	0.000
<i>HEX-2</i>	n.e.		0.100	0.095	0.091	0.047	0.099	0.237	0.177	0.161	0.000	0.000	0.046	0.043
<i>B-GUS</i>	n.e.		0.000	0.000	0.000	0.000	0.029	0.028	0.000	0.000	0.047	0.046	0.000	0.000
<i>MPI</i>	0.549	0.545	0.444	0.553	0.500	0.500	0.400	0.620	0.494	0.584	0.529	0.533	0.702	0.455
<i>GPI</i>	0.237	0.260	0.211	0.259	0.103	0.100	0.456	0.482	0.320	0.369	0.266	0.326	0.363	0.333

TABLE IV

Nei's (1972) indices of genetic distance D , above the diagonal and genetic identity I below the diagonal comparing *Balanus amphitrite amphitrite* from several localities and *B. amphitrite saltonensis*

	<i>B. a. saltonensis</i>		<i>B. a. amphitrite</i>			
	Salton Sea	Oakland	Mission B.	Bombay	Hong Kong	Singapore
<i>B. a. saltonensis</i>		0.001	0.002	0.003	0.003	0.003
<i>B. a. amphitrite</i>						
Oakland	0.999		0.003	0.026	0.005	0.027
Mission Bay	0.998	0.997		0.003	0.004	0.020
Bombay	0.977	0.975	0.977		0.014	0.004
Hong Kong	0.997	0.995	0.996	0.986		0.013
Singapore	0.977	0.974	0.980	0.996	0.987	

TABLE V

Nei's (1972) indices of genetic distance D , above the diagonal and genetic identity I below the diagonal, comparing *Balanus amphitrite amphitrite* from several localities and *B. variegatus*

	<i>Balanus amphitrite amphitrite</i>					<i>B. variegatus</i>
	Oakland	Mission B.	Bombay	Hong Kong	Singapore	Singapore
<i>B. a. amphitrite</i>						
Oakland		0.003	0.016	0.003	0.016	0.938
Mission Bay	0.997		0.014	0.002	0.010	0.935
Bombay	0.984	0.986		0.009	0.004	0.924
Hong Kong	0.997	0.996	0.991		0.008	0.918
Singapore	0.984	0.990	0.986	0.992		0.937
<i>B. variegatus</i>	0.392	0.392	0.397	0.399	0.392	

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ZUSAMMENFASSUNG

Balanus amphitrite ist eine außerordentlich variable Art innerhalb der balanomorphen Cirripedier. Aufgrund unvollständiger Diagnosen der früher beschriebenen Erscheinungsformen ergab sich eine beträchtliche Verwirrung in der Taxonomie der *B. amphitrite*-Gruppe. Durch elektrophoretische Analysen die bis zu 18 Enzymloci faßten, wurden die Indices der genetischen Identität bzw. Divergenz für drei Taxa innerhalb der *B. amphitrite*-Gruppe ermittelt. Die Ergebnisse führen zu dem Schluß, daß *Balanus amphitrite saltonensis* neu klassifiziert werden sollte als *Balanus amphitrite amphitrite*. *Balanus variegatus* konnte in seiner taxonomischen Stellung als Art bestätigt werden.

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