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Population Genetics of *Crepidula onyx*: Variation in a Californian Slipper Snail Recently Established in China

by

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Abstract. *Crepidula onyx* Sowerby, a common slipper snail on the coast of southern California, was first reported in Hong Kong Harbor in 1979. The genetic variation in and between three Californian populations and one Hong Kong population was determined using starch-gel electrophoresis. Seventeen enzyme systems were examined, yielding data on 23 presumptive loci in each individual studied. Sixteen loci were polymorphic in *C. onyx*. The percentage of polymorphic loci (P) ranged from 56.5 to 69.6%; mean heterozygosity (H_0) by direct count ranged from 0.14 to 0.18. Heterozygote deficiencies were found in all populations. For comparative purposes we also investigated the genetic variability in one population of *C. adunca* from central California.

An average genetic distance (Nei's unbiased D) between Hong Kong and California was 0.053. In contrast, the intersample D for the three Californian samples was 0.023. As the trans-Pacific D value was inflated by the inclusion of 10 alleles undetected in China we interpret the data as showing that the *Crepidula* introduced into Hong Kong are clearly derived from autochthonous Californian populations. The presence of six alleles found in Hong Kong and San Diego, but not at Balboa Island, suggest that the Chinese colonists were derived originally from the San Diego area rather than Los Angeles. Testing this hypothesis would require additional samples from the Los Angeles area and from Japan where *C. onyx* (not *C. fornicata*) became established in the 1960's.

INTRODUCTION

COLONIZING SPECIES afford us opportunities to study some of the major problems of theoretical ecology and evolutionary biology. Successful colonists reveal a great deal about population regulation, ecobehavioral aspects of habitat selection, interspecific interactions, and other coevolutionary phenomena (BAKER & STEBBINS, 1965; FUTUYMA & SLATKIN, 1983; PARSONS, 1983). Speciation may occasionally follow from genetic changes associated with the founder event and models of speciation by genetic revolution, founder-flush, and genetic transilience may be tested by observation of the colonization process (CARSON & TEMPLETON, 1984). Discussions of the proper taxonomic treatment of colonists and other disjunct populations permeate the systematic literature and the relevancy of the colonization process to the phyletic gradualism-punctuated equilibrium debate is increasingly clear. Yet, despite their considerable importance, we still lack a general theory of colonization that permits predictive statements about the success, failure, and biological impact of founding populations on the communities into which they are introduced. This conclusion was reached more than 20 years ago by ELTON (1958) and WILSON (1965) and is dramatically underscored by our experience with insects (REMINGTON, 1968; PRICE, 1980) and plants (BROWN & MARSHALL, 1981; CLEGG & BROWN, 1983). The same is true for mollusks; existing theory was inadequate to predict recent events involving the giant African land snail *Achatina fulica* (MEAD, 1961, 1979) and the Asiatic clam *Corbicula fluminea* (BRITTON, 1985) to name just two examples. Furthermore, as mollusks are an extraordinarily diverse group from a genetic viewpoint (SELANDER & OCHMAN, 1983) additional studies of a wide range of col-

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onists and noncolonists are needed before we can expect useful generalizations to emerge. For this reason we have been studying genetic aspects of various molluscan colonists including *Biomphalaria straminea* (WOODRUFF *et al.*, 1985a, b), *Cerion* spp. (WOODRUFF & GOULD, 1981, submitted), and *Achatina fulica* (Woodruff, in preparation). In the present paper we report on the population genetics of a Californian slipper snail that recently became established in China.

Crepidula onyx Sowerby is a common slipper snail on the coast of southern California. It ranges from southern California in the north to Chile in the south according to ABBOTT & HADERLIE (1980). However, earlier records of its southern range extent are suspect as the taxonomy of the central American *Crepidula* needs revision. Also, reports of *C. onyx* found north of California may be in error (Elaine Hoagland, personal communication, 1985). Within its range it is found on rocks, *Tegula* and mussel shells, pilings, and debris in the low intertidal and subtidal zones. Its life history was studied by COE (1942a) who found that this protandric hermaphrodite lived from 2 to 3 years in southern California. Following a two-week or longer pelagic larval stage the juveniles settle and grow quickly, attaining a shell length of 6–60 mm in one year. Snails with a shell length of 6 to 10 mm were functional males. Females were between 10 and 60 mm in length. Males were often found on the shells of females and, although COE (1942b) reports finding stacks of up to 17 snails, we have observed no more than three snails together. We can find no record of *C. onyx* occurring outside its natural range until, in 1968, it was noted (as *C. fornicata*) in Tokyo Bay, Honshu, Japan, by HABE & MAZE (1970). All references to *C. fornicata* in the Pacific are most likely misidentifications of *C. onyx* (Brian Morton, personal communication, 1985). *Crepidula onyx* subsequently spread south along the coast of Honshu and to the southern islands of Shikoku and Kyushu. In 1979 it was found on Hong Kong island by Dr. M. W. Yipp. Two years later a survey showed it was widespread in Hong Kong Harbor (Victoria Harbor) (HUANG *et al.*, 1983). Numerous earlier malacological surveys provide no evidence that it reached Hong Kong more than a few years before its discovery in 1979. We speculate that *C. onyx* arrived in this busy port in the mid-1970's either directly from California or, more likely, by way of Japan.

To investigate genetic aspects of the Chinese colonists we employed electrophoretic techniques to study allozymic variation (FERGUSON, 1980). Previous studies of allozyme variation in molluscan colonists have been very successful in elucidating the roles of demography, breeding system, and genetic drift in shaping the daughter populations (see Discussion). We were able to build on the contributions of HOAGLAND (1984) who established the basic patterns of genetic variability in *Crepidula*. She used starch-gel electrophoresis to resolve 24 loci in 7 species: *C. onyx* from California, *C. fornicata* from New England, *C. protea* from Brazil, and two pairs of sibling species presently referred to *C. plana* and *C. convexa* from the Atlantic coast of North America. From data published in the Appendix to her paper one can calculate the proportion of loci that are polymorphic in *C. onyx* to be $P = 0.435$. This relatively high value suggested that the species was variable enough to warrant this attempt to trace the colonization process geographically and monitor its genetic consequences in the derived populations. Further, HOAGLAND's (1984) demonstration that the various species she studied were very well differentiated from one another (Nei's $D \geq 0.39$) gave us reason to believe that we would be able to confirm the identity of the Chinese snails genetically. For comparative purposes we examined the population genetic variability in a second species of *Crepidula* from California; this is the first report on the genetics of *C. adunca* Sowerby.

MATERIALS AND METHODS

Specimens of *Crepidula onyx* were collected from three intertidal sites along the southern California coast in 1983 and from one site in Hong Kong:

1. The SAN DIEGO BAY site is a rocky bank adjacent to the Municipal Fishing Pier on Shelter Island. It is the southernmost collection site. *Crepidula onyx* was found on the surface of the rocks.

2. The MISSION BAY site is a rocky edge of a muddy channel located under the Ingraham Street traffic bridge, approximately 8 km north of the San Diego Bay site. Some *C. onyx* were found on mussels on the bridge pilings, although most were found on the surfaces of flat rocks.

3. The BALBOA ISLAND site (at Newport Beach, approximately 132 km north of San Diego) is a sandy beach with small boat piers, on the ocean side of the island, at the end of Jade Street. *Crepidula onyx* was found on mussels, on pilings, and on miscellaneous debris.

4. Dr. May Yipp provided us with a sample of *C. onyx* from North Point on the south shore of HONG KONG HARBOR (site F on fig. 1B of HUANG *et al.*, 1983).

Crepidula adunca was collected from a single intertidal site on the central California coast, at the southernmost end of Monterey Bay, also in 1983:

5. The ASILOMAR (central California) site is a rocky beach facing open ocean. *Crepidula adunca* was found on *Tegula* shells among tide pools.

Individuals were collected at each site during low tide using laboratory spatulas. Each collection consisted of approximately 40 individuals. All individuals were removed from the shells, sexed, and stored in centrifuge tubes at −70°C until electrophoresed. Voucher specimens are deposited with the California Academy of Sciences and Academy of Natural Sciences, Philadelphia.

Before electrophoresis, individual *Crepidula* were thawed, then homogenized in 0.1–0.2 mL of a grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP; pH 7.0). The homogenate was centrifuged at 10,000 *g* for

Table 1

Presumptive loci and electrophoretic conditions for *Crepidula onyx*.

Isozyme	No. alleles	Buffer	Voltage	Run time (hours)
Acid phosphatase (*Acp*)	1	TC 6.0	110 V	4.0
Alkaline phosphatase (*Alkp*)	3	TBE 9.0	250 V	4.0
Aspartate amino transferase (*Aat*)	3	TBE 9.0	250 V	4.0
Esterase-1 (*Es-1*)	3	TBE 9/8	210 V	2.5
Esterase-2 (*Es-2*)	3	TBE 9/8	210 V	2.5
Esterase-3 (*Es-3*)	1	TBE 9/8	210 V	2.5
Glucose-6-phosphate dehydrogenase (*G6pdh*)	4	Poulik	200 V	4.5
Glucose phosphate isomerase (*Gpi*)	3	TC 6.0	110 V	4.0
Hexokinase (*Hk*)	1	TBE 8.0	260 V	4.0
Isocitrate dehydrogenase (*Idh*)	1	TBE 8.0	260 V	4.0
Lactate dehydrogenase (*Ldh*)	3	TBE 9/8	210 V	2.5
Malate dehydrogenase-1 (*Mdh-1*)	2	TC 6.0	110 V	4.0
Malate dehydrogenase-2 (*Mdh-2*)	5	TC 6.0	110 V	4.0
Malic enzyme (*Me*)	1	TBE 9.0	250 V	4.0
Peptidase-1 (*Pep-1*) (leucylglycylglycine)	4	TBE 8.0	260 V	4.0
Peptidase-2 (*Pep-2*) (L-leucyl-L-alanine)	4	TBE 8.0	260 V	4.0
Peptidase-3 (*Pep-3*) (L-leucyl-L-alanine)	3	TBE 8.0	260 V	4.0
Phosphoglucomutase-1 (*Pgm-1*)	3	TC 6.0	110 V	4.0
Phosphoglucomutase-1 (*Pgm-1*)	3	TBE 9/8	210 V	2.5
Phosphoglucomutase-2 (*Pgm-2*)	5	TBE 9/8	210 V	2.5
6-Phosphogluconate dehydrogenase (*Pgd*)	3	TBE 8.0	260 V	4.0
Sorbitol dehydrogenase (*Sordh*)	3	TBE 9.0	250 V	4.0
Superoxide dismutase (*Sod*)	1	TBE 8.0	260 V	4.0
Xanthine oxidase (*Xdh*)	1	Poulik	200 V	4.5

2 min and then absorbed onto filter paper wicks which were inserted into 12.5% (w/v) horizontal starch gels (25 g Electrostarch Lot #392, 25 g Sigma starch/400 ml buffer).

Electrophoresis was carried out at constant voltage for approximately 4 h or until a bromophenol blue dye marker had migrated 10 cm from the origin. Electrophoretic conditions for the 17 enzymes are described in Table 1. Gels were stained at 37°C following the methods of SHAW & PRASAD (1970), SELANDER *et al.* (1971), HARRIS & HOPKINSON (1976), and HOAGLAND (1984). Esterases were stained using α-naphthyl-acetate as the substrate; peptidases used leucylglycylglycine or L-leucyl-L-alanine as substrate.

Isozymes were numbered in order of decreasing anodal mobility in multilocus systems. Relative mobilities of electromorphs at each locus were calculated using the most common allele at San Diego Bay as a standard. Typically several populations were run on each gel to facilitate comparison of alleles across populations.

The proportion of polymorphic loci (P) was based on direct count. Average heterozygosity per individual was based on direct count and Castle-Hardy-Weinberg expectations. For each polymorphic locus a χ^2 statistic was used to test the fit of the observed data to the random mating expectations of the Castle-Hardy-Weinberg model. Expected frequencies were calculated using LEVENE's (1949) correction for small samples. Exact significance probabilities were also calculated (analogous to Fisher's exact test for 2 × 2 contingency tables).

The genetic structure among the populations was analyzed using WRIGHT's (1978) F-statistics. The F_{is} value is the inbreeding coefficient of an individual relative to its sample. The F_{st} value is a measure of interdemic variation among samples relative to the total, and the F_{it} value provides a correlation between gametes combined within an individual relative to the total sample. A hierarchical analysis of population differentiation based on the F-statistics was performed using the partitioned variance equation: $H_T = H_L + D_{LA} + D_{AC} + D_{CT}$ (where T = total, L = locality, A = area, and C = country) and then setting $H_T = 1$ and partitioning each value. Unbiased estimates of genetic distance were calculated by the methods of NEI (1978) and ROGERS (1972). The distance measures for the five populations were clustered using UPGMA algorithms. Finally, Roger's distances were used to construct a Wagner tree. All these statistical analyses were carried out using the BIOSYS-1 program (SWOFFORD & SELANDER, 1981).

RESULTS

The 17 enzyme systems examined yielded data on 23 genes in each individual studied. Seven gene loci were monomorphic (*i.e.*, invariant) in all specimens of *Crepidula onyx*

Table 2

Allele frequencies for *Crepidula onyx* and *C. adunca*.

Locus	*Crepidula onyx* San Diego	Mission Bay	Balboa Island	Hong Kong	*C. adunca* Asilomar
Acp					
(n)	40	21	46	66	28
5.0	0.000	0.000	0.000	0.000	1.000
1.0	1.000	1.000	1.000	1.000	0.000
Alkp					
(n)	45	41	46	66	28
1.10	0.011	0.012	0.000	0.000	0.000
1.00	0.922	0.793	1.000	0.970	1.000
0.85	0.067	0.195	0.000	0.030	0.000
Aat					
(n)	43	40	38	53	27
1.25	0.000	0.000	0.000	0.000	0.574
1.20	0.000	0.000	0.000	0.000	0.426
1.13	0.163	0.050	0.013	0.170	0.000
1.00	0.558	0.600	0.697	0.642	0.000
0.81	0.279	0.350	0.289	0.189	0.000
Es-1					
(n)	45	40	45	65	26
1.10	0.044	0.125	0.033	0.000	0.231
1.05	0.400	0.538	0.744	0.777	0.019
1.00	0.556	0.338	0.222	0.223	0.346
0.94	0.000	0.000	0.000	0.000	0.404
Es-2					
(n)	44	41	46	66	27
1.00	0.818	0.573	0.837	0.242	0.926
0.95	0.182	0.415	0.163	0.652	0.074
0.91	0.000	0.012	0.000	0.106	0.000
Es-3					
(n)	46	43	46	66	28
1.00	1.000	1.000	1.000	1.000	0.000
0.89	0.000	0.000	0.000	0.000	1.000
G6pdh					
(n)	44	37	40	42	13
1.06	0.193	0.324	0.313	0.262	0.000
1.03	0.000	0.054	0.000	0.000	0.000
1.00	0.807	0.622	0.663	0.738	0.000
0.92	0.000	0.000	0.000	0.000	1.000
0.90	0.000	0.000	0.025	0.000	0.000
Gpi					
(n)	38	22	46	55	14
1.3	0.066	0.000	0.065	0.209	0.000
1.0	0.934	1.000	0.935	0.673	0.000
0.89	0.000	0.000	0.000	0.118	0.000
0.68	0.000	0.000	0.000	0.000	1.000
Hk					
(n)	44	40	36	65	28
1.00	1.000	1.000	1.000	1.000	1.000
Idh					
(n)	44	39	46	53	27
1.50	0.000	0.000	0.000	0.000	1.000
1.00	1.000	1.000	1.000	1.000	0.000

Table 2

Continued.

Locus	*Crepidula onyx* San Diego	Mission Bay	Balboa Island	Hong Kong	*C. adunca* Asilomar
Ldh					
(n)	46	43	46	66	28
1.10	0.000	0.000	0.000	0.000	1.000
1.05	0.207	0.093	0.000	0.197	0.000
1.00	0.793	0.814	1.000	0.576	0.000
0.98	0.000	0.093	0.000	0.227	0.000
Mdh-1					
(n)	46	40	46	66	28
1.15	0.098	0.025	0.022	0.015	0.000
1.00	0.902	0.975	0.978	0.985	0.000
0.77	0.000	0.000	0.000	0.000	1.000
Mdh-2					
(n)	44	40	42	36	28
2.4	0.034	0.000	0.060	0.014	0.000
2.0	0.386	0.438	0.286	0.528	0.000
1.5	0.045	0.087	0.131	0.167	0.000
1.0	0.523	0.438	0.381	0.250	0.000
0.5	0.011	0.038	0.143	0.042	0.000
0.4	0.000	0.000	0.000	0.000	1.000
Me					
(n)	46	42	46	62	27
1.30	0.000	0.000	0.000	0.000	1.000
1.00	1.000	1.000	1.000	1.000	0.000
Pep-1					
(n)	46	40	44	60	28
1.12	0.054	0.063	0.170	0.192	0.268
1.04	0.348	0.338	0.375	0.417	0.304
1.00	0.489	0.412	0.307	0.267	0.071
0.84	0.109	0.188	0.148	0.125	0.357
Pep-2					
(n)	40	38	46	52	28
1.00	0.675	0.329	0.717	0.654	0.964
0.95	0.150	0.118	0.000	0.000	0.036
0.92	0.175	0.553	0.239	0.346	0.000
0.76	0.000	0.000	0.043	0.000	0.000
Pep-3					
(n)	45	34	46	31	28
1.35	0.000	0.000	0.000	0.000	0.054
1.25	0.000	0.000	0.000	0.000	0.946
1.00	0.489	0.309	0.609	0.919	0.000
0.95	0.100	0.324	0.000	0.000	0.000
0.90	0.411	0.368	0.391	0.081	0.000
Pgm-1					
(n)	37	27	42	61	
1.08	0.392	0.167	0.131	0.205	
1.00	0.419	0.593	0.429	0.270	
0.91	0.189	0.241	0.440	0.525	
Pgm-2					
(n)	24	20	46	33	28
1.15	0.021	0.150	0.000	0.000	0.000
1.10	0.000	0.050	0.000	0.000	0.000

Table 2

Continued.

Locus	*Crepidula onyx* San Diego	Mission Bay	Balboa Island	Hong Kong	*C. adunca* Asilomar
1.00	0.917	0.775	0.880	0.970	0.000
0.91	0.042	0.025	0.076	0.000	0.000
0.90	0.021	0.000	0.043	0.030	0.000
0.79	0.000	0.000	0.000	0.000	0.518
0.70	0.000	0.000	0.000	0.000	0.482
Pgd					
(n)	44	37	46	47	14
1.17	0.034	0.000	0.000	0.043	0.000
1.00	0.966	0.986	1.000	0.915	1.000
0.75	0.000	0.014	0.000	0.043	0.000
Sordh					
(n)	44	42	43	54	28
1.88	0.205	0.036	0.221	0.343	0.036
1.25	0.341	0.321	0.070	0.435	0.000
2.00	0.455	0.643	0.709	0.222	0.964
Sod					
(n)	42	37	42	26	
1.00	1.000	1.000	1.000	1.000	
Xdh					
(n)	46	43	45	59	28
1.00	1.000	1.000	1.000	1.000	1.000
Percentage of loci polymorphic (*P*)	69.6	65.2	56.5	69.6	34.8
Mean heterozygosity (*H*)	0.156	0.176	0.169	0.141	0.052

examined: *Acp, Es-3, Hk, Idh, Me, Sod,* and *Xdh.* Sixteen loci were polymorphic: one locus had two alleles (*Mdh-1*), 10 loci had three alleles (*Alkp, Aat, Es-1, Es-2, Gpi, Ldh, Pep-3, Pgm-1, Pgd, Sordh*), three loci had four alleles (*G6pdh, Pep-1, Pep-2*), and two loci had five alleles (*Mdh-2, Pgm-2*). In *C. adunca,* no data were obtained for *Pgm-1* and *Sod.* Of the remaining loci, six had two alleles (*Aat, Es-2, Pep-2, Pep-3, Pgm-2, Sordh*), two had three alleles (*Es-1, Pep-1*), and the remainder were monomorphic. The relative mobilities of these allozymes are shown in Table 2. The allele frequencies for all loci in *C. onyx* and *C. adunca* are also shown in Table 2 along with values for *P* (percentage of polymorphic loci) and *H* (mean heterozygosity).

In *Crepidula onyx* it can be seen that *P* has a range of 56.5 to 69.6%; *C. adunca* has a lower value of 34.8%. Mean heterozygosity by direct count ranges from 0.14 to 0.18 in *C. onyx,* and again *C. adunca* has a lower value (0.05). These values consistently underestimate intrapopulation *H* as the populations of adult snails sampled all show consistent heterozygote deficiencies (see below). Mean heterozygosities according to panmictic expectations were 0.24–0.30 in *C. onyx* and 0.12 in *C. adunca.*

The data were examined for evidence of panmixia, and heterozygote deficiencies were found in all populations (Table 3). There are 16 loci in the four populations of *Crepidula onyx* at which observed allele frequencies can be compared with those expected on the basis of panmixia. Simple χ^2 tests on the raw genotypic data showed that out of 60 tests, fully 40 failed at the 0.05 level. However, because of inadequacies of the χ^2 test, we chose to calculate exact significance probabilities (Table 4). In San Diego Bay, it can be seen that significance tests for 11 out of 16 loci fail using this procedure. For comparison, in Mission Bay 7 out of 15, at Balboa Island 6 out of 13, and in Hong Kong Harbor 9 out of 16 loci all show significant heterozygote deficiencies. *Crepidula adunca* (the outgroup) shows the same pattern. Looking at Table 4 by specific locus, it can be seen that *Es-1, G6pdh, Ldh,* and *Pgm-1* are out of equilibrium in all populations and *Es-2* is out at all populations except Asilomar. Only *Mdh-1* and *Pep-lgg-1* are segregating according to panmictic expectations in all populations; *Mdh-2* is in equilibrium at all populations except Hong Kong.

Values for Wright's F-statistics are presented in Table 5. The mean F_{is} value was 0.415 and is significantly different from zero; F_{st} was 0.079 and the F_{it} value was 0.461.

A matrix of genetic similarity and distance coefficients is shown in Table 6. The UPGMA phenogram resulting from Nei's genetic identity is shown in Figure 1; its cophenetic correlation coefficient was 0.999. A similar phenogram resulted from Rogers' distance with San Diego Bay, Balboa Island, Mission Bay, and Hong Kong each having distances of approximately 0.12 and Asilomar having a distance of 0.58. The correlation was 0.998. The Rogers distance values were used to prepare the Wagner tree shown in Figure 2.

DISCUSSION

We are confident that the dark-shelled Chinese snails we have studied are correctly identified as *Crepidula onyx.* Identifications based on morphology (CHRISTIAENS, 1980; HUANG *et al.,* 1983; Hoagland, personal communication, 1983) are herein confirmed genetically. The only other calyptraeoid snail known from the Hong Kong area is *Crepidula* (*Siphopatella*) *walshi* (Reeve) and the two species differ in gross appearance and habit (CHRISTIAENS, 1980). *Crepidula walshi* is white-shelled and commensal on the inner surface of gastropod shells occupied by pagurid hermit crabs (YIPP, 1980).

Autochthonous populations of *Crepidula onyx* from southern California are genetically variable. Our study of variation at 23 loci in 135 animals representing 3 populations gave an estimate of the mean proportion of polymorphic loci (*P*) of 0.637. If we add to these data, the estimate of $P = 0.565$ for 58 animals from Balboa Island published by HOAGLAND (1984; identical to our estimate) then we obtain a value for *C. onyx* of $P = 0.619$. Com-

Table 3

Coefficients for heterozygote deficiency or excess in all polymorphic loci.

	San Diego			Mission Bay			Balboa Island			Hong Kong			Asilomar		
Locus	Obs. hets	Exp. hets	*D*	Obs. hets	Exp. hets	*D*	Obs. hets	Exp. hets	*D*	Obs. hets	Exp. hets	*D*	Obs. hets	Exp. hets	*D*
Alkp	3	6.6	−0.55	11	13.8	−0.21	—	—	—	4	3.9	0.02	—	—	—
Aat	13	25.4	−0.49	10	20.9	−0.52	11	16.5	−0.34	19	28.0	−0.32	5	13.5	−0.63
Es-1	6	24.1	−0.75	13	23.6	−0.45	4	18.0	−0.78	5	22.7	−0.78	5	17.6	−0.72
Es-2	6	13.2	−0.55	11	20.7	−0.47	3	12.7	−0.76	6	33.6	−0.82	4	3.8	0.06
G6pdh	9	13.9	−0.35	4	19.0	−0.79	11	18.7	−0.41	2	16.4	−0.88	—	—	—
Gpi	5	4.7	0.06	—	—	—	4	5.7	−0.30	12	27.2	−0.56	—	—	—
Ldh	5	15.2	−0.67	4	13.9	−0.71	—	—	—	6	38.4	−0.84	—	—	—
Mdh-1	7	8.2	−0.15	2	2.0	0.01	2	2.0	0.01	2	2.0	0.01	—	—	—
Mdh-2	23	25.6	−0.10	23	24.6	−0.07	27	31.1	−0.13	16	23.0	−0.30	—	—	—
Pep-1	27	29.1	−0.07	25	27.4	−0.09	30	31.8	−0.06	28	42.5	−0.34	13	20.1	−0.35
Pep-2	4	19.9	−0.80	15	22.0	−0.32	12	19.8	−0.40	22	23.8	−0.07	0	2.0	−1.00
Pep-3	14	26.5	−0.47	12	22.9	−0.48	22	22.2	−0.01	5	4.7	0.07	1	2.9	−0.65
Pgm-1	10	23.8	−0.58	4	15.5	−0.74	20	25.7	−0.22	20	37.5	−0.47	—	—	—
Pgm-2	4	3.9	0.04	3	7.7	−0.61	11	10.1	0.09	2	2.0	0.02	5	14.2	−0.65
Pgd	1	2.9	−0.66	1	1.0	0.00	—	—	—	0	7.6	−1.00	—	—	—
Sordh	16	28.3	−0.43	17	20.5	−0.17	11	19.3	−0.43	21	35.1	−0.40	0	2.0	−1.00

parable data are available for seven other species of *Crepidula* (HOAGLAND, 1984). Over all eight species, studied at 21–24 loci, estimates of *P* range from 0.348 in *C. adunca* (this study) to 0.875 in *C. fornicata*. The grand mean $P = 0.468$ (SD = 0.287).

The Californian samples of adult *Crepidula onyx* we studied have a mean heterozygosity by direct count (*H*) of 0.167. This value is, however, not simply comparable to the mean value of $H = 0.148$ for 46 other molluscs published by NEVO *et al.* (1984) as we have detected a consistent pattern of heterozygote deficiency in *C. onyx*. As our value underestimates *H*, *C. onyx* falls into the "above-average" class for levels of individual genetic variability in mollusks. Turning now to the allochthonous (and probably adventive) Chinese snails we estimate $P = 0.696$ and $H = 0.141$ (Table 2). These values are very similar to those found in the Californian samples. Again, *H* is an underestimate of individual variability.

The rich genetic endowment of *Crepidula onyx* held the promise that we might be able to reconstruct the colonization process by careful comparison of the Californian and Chinese snails. Multilocus comparisons of the samples from the two areas do, in fact, confirm the genetic relatedness of the populations now over 11,000 km apart. Estimates of overall genetic differentiation based on formulae developed by NEI (1978) give an average genetic distance (*D*) between Hong Kong and California of 0.053 (range 0.049–0.058). In contrast, the intersample *D* for the three Californian samples is 0.023 (0.021–0.027). In mammals, fish, reptiles, and amphibians interspecific variation is such that congeneric populations of outcrossing species typically have *D* values of 0.10 or more (AVISE & AQUADRO, 1982). The average genetic distance between local populations for a wide range of plants, invertebrates, and vertebrates ranges between 0.013–0.058 (AYALA, 1983). As the trans-Pacific *D* value is inflated by the inclusion of 10 Californian alleles that apparently did not become established in China, we interpret the data as showing that the Asian snails are clearly derived from, and poorly differentiated from, their American ancestors. Thus, despite its recent range expansion, *C. onyx* is not more differentiated than other species of *Crepidula* where intraspecific differences are in the range: 0.003–0.016 (*C. fornicata*), 0.008–0.076 (*C.* cf. *convexa*), 0.037–0.057 (*C. convexa*), 0.052–0.097 (*C. plana*), and 0.045 (*C.* cf. *plana*) (HOAGLAND, 1984).

San Diego (samples from San Diego Harbor and nearby Mission Bay) and Balboa Island at Newport Beach, about 30 km from the port of Los Angeles, are representative of the two major commercial and naval ports within the range of *Crepidula onyx*. Accordingly, we applied a variety of clustering techniques to the multilocus data in an attempt to establish whether the Chinese population was more closely related to San Diego or Balboa Island, California populations. A phenogram, based on the UPGMA method using Nei's *D* (Figure 1) and a Wagner tree based on Rogers' *S* (Figure 2) showed that the Chinese population was equidistant from the three Californian populations sampled, or (insignificantly) closer to Balboa Island than San Diego. These analyses are, however, misleading as they do not allow for the known historical relationship between the Asian and American snails. A closer analysis of the original data (Table 2) suggests a different conclusion—one that points to San Diego as the likely source of the colonists. We detected 58 alleles segregating in Californian *C. onyx* and 48 of these were found

Table 4

Exact significance probabilities of agreement with Hardy-Weinberg expectations.

Locus	San Diego	Mission Bay	Balboa Island	Hong Kong	Asilomar
Alkp	0.013	0.330	—	1.000	—
Aat	0.001	0.000	0.059	0.072	0.001
Es-1	0.000	0.005	0.000	0.000	0.000
Es-2	0.002	0.004	0.000	0.000	1.000
G6pdh	0.035	0.000	0.029	0.000	—
Gpi	1.000	—	0.159	0.000	—
Ldh	0.000	0.000	—	0.000	—
Mdh-1	0.351	1.000	1.000	1.000	—
Mdh-2	0.237	1.000	0.207	0.008	—
Pep-1	0.081	1.000	0.332	0.064	0.240
Pep-2	0.000	0.114	0.024	0.759	0.018
Pep-3	0.016	0.077	1.000	1.000	0.055
Pgm-1	0.000	0.000	0.026	0.002	—
Pgm-2	1.000	0.023	1.000	1.000	0.001
Pgd	0.034	1.000	—	0.000	—
Sordh	0.003	0.314	0.021	0.002	0.018

Table 5

Summary of F-statistics for *Crepidula onyx*.

Locus	F_{is}	F_{it}	F_{st}
Aat	0.415	0.427	0.020
Acp	1.000	1.000	0.084
Alkp	0.264	0.323	0.081
Es-1	0.663	0.693	0.087
Es-2	0.644	0.716	0.201
Es-3	1.000	1.000	0.018
G6pdh	0.619	0.626	0.020
Gpi	0.406	0.475	0.117
Ldh	0.761	0.788	0.111
Mdh-1	0.073	0.100	0.029
Mdh-2	0.141	0.170	0.034
Me	−0.025	−0.012	0.012
Pep-1	0.132	0.149	0.019
Pep-2	0.393	0.449	0.092
Pep-3	0.303	0.411	0.154
Pgd	0.802	0.808	0.027
Pgm-1	0.493	0.527	0.065
Pgm-2	0.236	0.274	0.049
Sod	0.725	0.733	0.027
Sordh	0.359	0.428	0.107
Mean	0.415	0.461	0.079

in Hong Kong. Ten Californian alleles were not seen in the 26–66 Chinese animals studied. These alleles varied in frequency in California between 0.012 and 0.324 ($\bar{X}$ = 0.101); it seems most probable that they were lost by chance during the colonization process. Of the 48 Californian alleles segregating in Hong Kong six were found in San Diego and not at Balboa Island: $Alkp^{0.85}$, $Es\text{-}2^{0.91}$, $Ldh^{1.05}$, $Ldh^{0.98}$, $Pgd^{1.17}$ and $Pgd^{0.75}$. Combining the Mission Bay and San Diego Harbor data, these six alleles had an estimated average frequency of 0.07 (range 0.01–0.21) in California and 0.11 (0.04–0.23) in China. As these six San Diego markers were not found at Balboa Island their presence in Hong Kong strongly suggests that the Chinese colonists were derived originally from San Diego Harbor rather than Los Angeles. Although our sampling of *C. onyx* is inadequate to prove this assertion, it is a reasonable hypothesis on the basis of existing data.

The phenograms based on the allozyme data obscure this pattern because they do not allow for the known derivation of the Chinese population from America. The UPGMA methods overemphasize similarities in allele frequencies and, in this respect, Hong Kong is rather like the Balboa Island sample, *e.g.*, $Pep\text{-}2^{0.95}$, $Pep\text{-}3^{0.95}$, and $Es\text{-}1^{1.05}$. The method ignores the fact that we would expect a sequential loss of rarer alleles during the population bottleneck(s) associated with the colonization process. The observation that six alleles associated only with San Diego and none associated exclusively with Balboa Island are now established in Hong Kong suggests that one can reach more definitive conclusions than those based on standard analytical procedures.

It should not surprise us that it is difficult to trace the origin of the Hong Kong stock back to America. *Crepidula onyx* is very variable and a hierarchical analysis of F-statistics reveals that fully 93% of the detected variation resides in any single sample. Less than 6% of the known genetic variation in the species is partitioned between populations. Even if we had multiple samples from Los Angeles and elsewhere it is unlikely that we could ever prove that the Chinese snails arose from one single region of the original species range. In fact, we already know that our sampling in California was unequal to this task as we discovered $Gpi^{0.89}$ at a frequency of 0.118 in Hong Kong and have yet to find this allele in America. (The alternative hypothesis, that this allele arose by post-colonization mutation is unlikely in view of the fact that snails have been in Hong Kong for only about 10 generations.)

One of the problems with deciphering the history of Hong Kong *Crepidula onyx* arises from the fact that we do not know whether the colonists came from California

Table 6

Matrix of genetic similarity and distance coefficients for *Crepidula onyx* and *C. adunca*. Below diagonal: Rogers' (1972) genetic distance. Above diagonal: Nei's (1978) unbiased genetic identity.

	C. onyx			*C. adunca*	
Population	1	2	3	4	5
1 San Diego Bay	*****	0.978	0.980	0.948	0.413
2 Ingraham Bridge	0.102	*****	0.973	0.944	0.401
3 Balboa Island	0.093	0.109	*****	0.952	0.421
4 Hong Kong	0.139	0.151	0.122	*****	0.615
5 Asilomar	0.572	0.585	0.563	0.361	*****

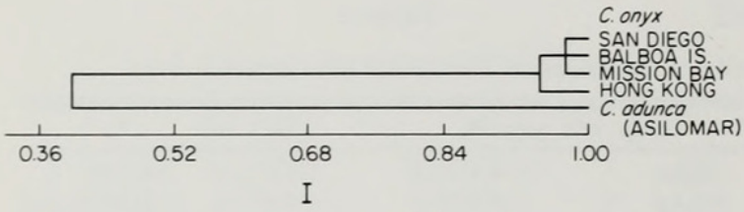

Figure 1

Genetic relationships revealed by UPGMA cluster analysis based on NEI's (1978) genetic identity.

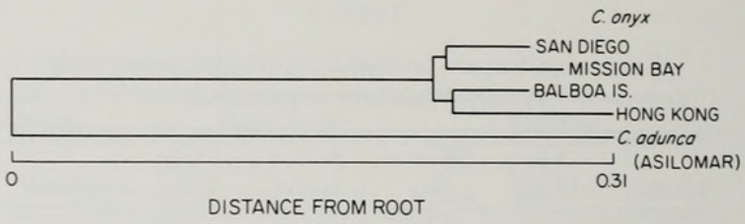

Figure 2

Cluster relationships of samples revealed by an outgroup rooted Wagner tree based on Rogers' genetic distance.

directly or indirectly by way of Japan. A survey of genetic variability among Japanese *C. onyx* may clarify this issue.

A second problem arises because *Crepidula onyx* is so polymorphic that single samples may not adequately represent its geographic and temporal variability. A comparison of our results (Table 2) for *C. onyx* from Balboa Island with those published by HOAGLAND (1984) reveals identical or insignificantly different allele frequencies at five of the 11 loci studied in both laboratories, but significant differences at the remaining six loci. The latter are probably not due to technical differences in our procedures but, rather, reflect the considerable geographic and temporal variability we have recently discovered by monitoring selected populations of this species over a 2-yr period (MCMEEKIN, 1985; MCMEEKIN & WOODRUFF, in preparation).

We can be a little more confident in our reconstruction of the colonization event. It is most probable that the founding population was not very small and that once established it grew rapidly. The high levels of individual heterozygosity (H) and the high overall value for P in the Hong Kong sample indicate no major bottleneck occurred. Such a scenario would account for the preservation of most of the ancestral variability (NEI *et al.*, 1975) and is the most frequently observed result of molluscan colonization (SELANDER & OCHMAN, 1983). As there are up to five alleles segregating per locus in Hong Kong there could be no fewer than three founders; there were probably at least 10 times that many. Once settled in Hong Kong there is no doubt that the population could grow very rapidly. *Crepidula onyx* is a protandrous hermaphrodite that can begin egg laying within two months of settling and can produce 5000–20,000 larvae during its 2–3-yr life (COE, 1942a). Ten or more generations of such growth in Hong Kong, coupled with dispersal during the 2-wk planktonic larval stage, would account for the present abundance and distribution of the species in this part of China. It is unnecessary, at this time, to postulate that multiple introductions have occurred, although it is quite likely that they have.

We find no obvious evidence for any major change in the population structure of the Hong Kong colonists. The mean number of alleles per locus (2.2) is not different from that seen in California (2.1–2.3). As noted above, the proportion of polymorphic loci (0.696 in Hong Kong *vs.* 0.619 in California) and the mean heterozygosity (0.167 *vs.* 0.141) are also very similar. A decrease in genetic variability has not occurred and we find no evidence for a genetic revolution of the type envisaged by MAYR (1954) associated with this founder event. Whether the Chinese populations will diverge from their ancestors according to the founder-flush or genetic transilience models (CARSON & TEMPLETON, 1984; BARTON & CHARLESWORTH, 1984) is more difficult to rule out given our present knowledge of the *Crepidula* genome. It does, however, seem most unlikely as future gene flow from America would retard local differentiation. Nevertheless, it would be interesting to examine the reweighting of fitness components due to the changes in allele frequencies associated with the founder event. If such genetic drift-induced shifts involve major loci with many pleiotropic effects, then the stage is set for transilience to occur.

Crepidula onyx resembles a number of colonizing species of plants and animals in having a richly variable genome that appears resistant to significant change by founder effect. BAKER (1965) suggested such species have a general purpose genotype that is resistant to repatterning. These "weedy" species are alleged to have complex balanced systems of heterosis that can survive bottlenecks intact and may even underlie the colonizing habit. Although we feel it is too early to state that such colonizing species have genetic properties that distinguish them from "speciose" species, it seems most likely that *C. onyx* fits the above pattern. We foresee no nomenclatural problems arising from the success of the Asian colonists; they seem likely to remain genetically very similar to their allopatric ancestors.

The most striking thing about the population structure of the Chinese *Crepidula onyx* is, of course, the striking heterozygote deficiencies at 11 of the 16 polymorphic loci. One might seize on this observation as evidence for dramatic genetic restructuring of the colonizing population. This, however, cannot be the explanation, as the same phenomenon occurs in the three Californian samples.

Over two dozen studies of genetic variability in marine mollusks have shown heterozygote frequencies lower than expected under panmixia (discussed by ZOUROS & FOLTZ, 1984). SINGH & GREEN (1984) propose four possible explanations for this phenomenon: inbreeding, presence of null alleles, Wahlund effect, and selection. In addition,

the explanations of scoring bias (AYALA *et al.*, 1973) and sex-linked loci (ZOUROS *et al.*, 1980) have been offered to account for this deficiency of heterozygotes.

The present paper was not aimed at resolving this issue; however, it can be shown that three of the above hypotheses are unlikely to account for the heterozygote deficiency in this study. First, inbreeding as an explanation is not compatible with the species' biology. *Crepidula* have a dispersed larval stage for at least two weeks, making it improbable for gametes from closely related individuals to combine in high frequencies. Because *Crepidula onyx* are sequential hermaphrodites, and are usually not sexually functional during the transition stage from male to female (COE, 1942a), self-fertilization is not a factor. Second, null alleles are unlikely to cause the heterozygote deficiency, as co-dominant null alleles would be recognizable as "blank" spots on the gel and would not be expected at such high frequency. No evidence for this has been seen in this study. Third, the Wahlund effect does not explain the deficiency, as this is not a local problem; relatively low heterozygote frequencies are found in all of the populations. We are at present conducting a study of *C. onyx* in Mission Bay, San Diego, which will, among other questions, assess the relative role of natural selection, age effects, sex-linked loci, and scoring bias in explaining the observed heterozygote deficiencies.

The same phenomenon of heterozygote deficiency was also noted in our sample of *Crepidula adunca* (a species with brooded larvae) from central California and it may be a general feature of slipper snails. In other respects *C. adunca* seems quite different from *C. onyx*. Genetically it is less variable: $A = 1.5$ *vs.* 2.3 where A is the number of alleles per locus, $P = 0.348$ *vs.* 0.619 and $H = 0.052$ (SE = 0.023) *vs.* 0.167; it is in fact less variable than any of the six Atlantic species studied by HOAGLAND (1984). Although additional samples are needed to confirm this result the two Californian species seem to have very different population genetic structures. *Crepidula adunca* and the California samples of *C. onyx* are also well differentiated from one another: NEI's (1978) $D = 0.888$ (range 0.884–0.913) (I = 0.401–0.421) and ROGERS' (1972) $D =$ 0.563–0.585. These distances are a little greater than those found between the six Atlantic species which had Nei's D of 0.31–0.87, with most comparisons falling in the range of 0.65–0.80 (HOAGLAND, 1984). Although our studies are not completely comparable it is interesting to note that HOAGLAND (1984) also estimated the genetic distance between *C. onyx* and the six Atlantic taxa: *C. fornicata,* 0.70–0.71; *C. convexa* and its unnamed sibling, 0.79–0.84; *C. plana* and its unnamed sibling, 0.76–0.82; and Brazilian *C. protea,* 0.72. Clearly, the geographic proximity of *C. onyx* and *C. adunca* belies their genetic unrelatedness and our observation that the two species differ markedly in genetic variability should not surprise us.

As a genetically variable and successful colonist *Crepidula onyx* is reminiscent of *C. fornicata,* which has spread from the Atlantic coast of North America to Europe (HOAGLAND, 1985). *Crepidula fornicata* is the most variable *Crepidula* yet described (HOAGLAND, 1984, 1985). As it forms long breeding chains of stacked individuals it is regarded as a fouling organism in some areas. ORTON (1912) surmised how this habit contributes to their success in oyster beds by not only increasing the chance of copulation but also by enhancing feeding and respiratory efficiency. *Crepidula onyx* also forms stacks and, although it is not presently a nuisance in California harbors and oyster beds, its habits in exotic locales deserve some attention. Its ecological impact in the warmer waters of Hong Kong might be expected to be greater than that along the coast of Japan. One thing is certain; with 10,000 ships entering Hong Kong waters each year the species will undoubtedly spread to other areas.

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