The Veliger 33(4):325–338 (October 1, 1990)

THE VELIGER

Reproductive, Morphological, and Genetic Evidence for Two Cryptic Species of Northeastern Pacific *Nucella*

by

A. RICHARD PALMER[1]

Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9, and
Bamfield Marine Station, Bamfield, British Columbia, Canada V0R 1B0

SCOTT D. GAYRON[2] AND DAVID S. WOODRUFF

Department of Biology C-016 and Center for Molecular Genetics, University of California San Diego,
La Jolla, California 92093, USA

Abstract. Laboratory crosses among four geographically distant populations of the northeastern Pacific, rocky shore gastropod *Nucella emarginata* (=*Thais emarginata*) (Deshayes, 1839) yielded evidence of reproductive isolation between "northern" and "southern" populations. Although between-population crosses with individuals from Torch Bay (Alaska), Bamfield (British Columbia), and San Juan Island (Washington) yielded viable F1 and F2 offspring, those between-population crosses involving individuals from Santa Barbara (California) yielded only capsules whose eggs did not develop. The success of within-population crosses for all four populations confirmed that this inability to reproduce was not a laboratory artifact. Northern and southern populations also differed in egg-capsule morphology (northern—vase shaped; southern—rolling-pin shaped) and in the form of spiral sculpture (northern—uniform spiral ribs; southern—regularly spaced knobs along the spiral ribs). Finally, a survey of allozyme variation revealed fixed allelic differences at one locus, and a genetic distance estimate (*D*) of 0.16, between two nearly sympatric populations separated by less than 500 m in central California. Collectively, these data suggest very strongly that northern and southern populations currently referred to *N. emarginata* actually belong to two reproductively isolated cryptic species.

INTRODUCTION

Because of their highly variable shells, thaidine gastropods from the northeastern Pacific have a colorful taxonomic history. Among the four currently accepted species, DALL (1915) listed no less than nine synonyms for *Nucella lamellosa,* five synonyms for both *N. lima* and *N. canaliculata,* and four for *N. emarginata.* In contrast, in apparent frustration because of their variable shells, TRYON (1880) had previously lumped all the currently recognized species of *Nucella* from the northern hemisphere into a single, geographically widespread species, *Nucella lapillus* [as *Purpura*]: " The quantity and variety of material before me, embracing a rich series of forms from many localities, together with the comparison of numerous descriptions and figures that have been published, induce me to include under this, the oldest name, a very large number of nominal species. . . . I have considered it preferable to retain some of these names as indicating growth modifications and localities; those who take a more conservative view than myself will thus have the names and descriptions at hand to designate these several forms as varieties or species, or even genera, if it so pleases them." More recently, KINCAID (1964) lumped the nominal species *canaliculata, emarginata,* and *lima* into the single species *Nucella lima,* because he considered their shells to intergrade. Clearly, traits other than shell form are required to distinguish among these and related species.

Each of the four currently recognized species of *Nucella* from the northeastern Pacific appears to have a wide geographic range (ABBOTT, 1974; PALMER, 1984a). Although some of these reported ranges are incorrect (VERMEIJ *et al.,* 1990), each species occurs over at least 5000 km of

[1] To whom reprint requests should be addressed.

[2] Present address: Department of Anthropology, 34 Haines Hall, University of California, Los Angeles, CA 90024, USA.

coastline. Furthermore, all four species lack a planktonic larval stage: crawl-away hatchlings emerge directly from the egg capsule (STRATHMANN, 1987). These wide geographic ranges, coupled with the presumably limited gene flow associated with the lack of pelagic larvae, should promote genetic divergence. Whether such divergence would be sufficient to result in reproductive isolation depends on its form and extent.

Low levels of gene flow do appear to have promoted local genetic differentiation in *Nucella lamellosa*. For example, GRANT & UTTER (1988) observed significantly different allozyme frequencies among populations on the scale of meters to hundreds of kilometers. However, based upon a survey of allozyme variation among 12 populations ranging from Glacier Bay, Alaska, to San Francisco, California, CAMPBELL (1978) concluded that in spite of extensive differences "there is no reason to assign the populations subspecific, racial or ecotypic status." Nonetheless, by themselves, differences in allozyme frequencies over large geographic distances reveal little about the potential viability or fecundity of hybrids, and hence provide at best a coarse estimate of a species' status.

When hybrid crosses are inviable, a convincing case can be made for cryptic species. Such crosses, however, are often difficult and time consuming, and do not permit many populations to be examined. Furthermore, reproductive isolation observed between geographically distant populations could result from isolation by distance (WRIGHT, 1943) or reflect the presence of two discrete species. In such a situation, a combination of genetic, ecological, and morphological variation among intervening populations would help delineate more clearly between these two alternatives. We present such evidence here.

COLLECTION SITES AND PROCEDURES

Reproductive Isolation and Morphological Variation

To test for reproductive isolation, individuals of *Nucellla emarginata* were collected from four sites along the Pacific coast of North America:

(I) TORCH BAY, ALASKA (code = AK)—the bedrock shores at the mouth of the west arm (58°19′41″N, 136°47′56″W), on the outer coast of the Glacier Bay National Monument in both July 1980 and July 1982,

(II) BAMFIELD, BRITISH COLUMBIA (code = BC)—the north shore of Wizard Id. (48°51′30″N, 125°09′33″W), near the Bamfield Marine Station on the west coast of Vancouver Id. in August 1980 and December 1981,

(III) SAN JUAN ISLAND, WASHINGTON (code = WA)—from among boulders on the north shore of Cattle Pt. (48°27′42″N, 122°58′58″W), 2–300 m E of the mouth of Jaekel's Lagoon in May 1981, and

(IV) SANTA BARBARA, CALIFORNIA (code = CA)—from the rocks of Coal Oil Pt. (34°23′N, 119°42′W), near the UCSB campus in December 1981.

Both within- and between-population crosses were initiated with field-collected animals. Snails were sexed and maintained in the laboratory as described in PALMER (1985a). Cages were checked for egg capsules every two to four weeks. Crosses were monitored for 12 to 34 months (mean = 18.9 months). All surviving parents from between-population crosses involving California snails were subsequently re-paired with snails from their source population to verify that they were fertile. These crosses were held and monitored as above for 13 to 23 months (mean = 17.4 months).

In any such study of reproductive isolation, the possibility that field-collected females store sperm must be addressed, particularly in gastropods where sperm may be stored for periods up to 12 months or more (ANSELL, 1982; FRETTER & GRAHAM, 1962). Except where noted, the 55 initial crosses in the present study were begun with immature snails (males: 16.7 ± 3.11 mm, females: 17.0 ± 3.14 mm; mean shell length ± SD, n = 47 both sexes). In six of the eight crosses started with mature female snails, at least six months were allowed to pass before egg capsules were collected (see Table 2a, b below) to allow for loss of stored sperm (PALMER, 1985a, and unpublished). In the remaining two crosses with mature female snails, capsules were collected less than six months after initiation (82-47, 82-49), but these capsules were saved specifically as part of a sperm-storage study using color markers. In both these cases, sperm from prior mating was no longer present after the first three clutches had been laid, and most subsequent clutches (five and six, respectively) were viable. In the fertility-test crosses, all the mature females were those from crosses in which none of the egg capsules yielded viable embryos; hence, stored sperm was not a consideration.

To determine the geographic distribution of distinctive features of spiral sculpture and egg-capsule morphology, snails and egg capsules were collected from more than 20 sites along the Pacific coast from Bamfield, BC, to Santa Barbara, California, in April and May 1985 (see Figure 3 below). An additional collection was made at Crystal Cove, California, in February 1990.

Allozyme Variation

Allozyme variation was examined in samples collected in April 1986 from five central California sites. These sites spanned the region where northern and southern forms of spiral sculpture and egg-capsule morphology appeared to overlap. One additional sample from Bamfield, collected in June 1986, was also analyzed for comparison with the California samples. Samples were collected as follows (from north to south):

BAMFIELD (48°50′04″N, 125°08′47″W)—from a continuous bedrock shore at the S end of Scott's Bay, near the Bamfield Marine Station (moderately exposed),

BODEGA HEAD (38°18′N, 123°04′W)—from the rocks near the southernmost tip (very exposed),

BODEGA BAY HARBOR (38°19′N, 123°03′W)—from among rock and concrete rubble along the W side of the channel inside the breakwater (very protected),

FORT PT. (37°49′N, 122°28′W)—from the boulders and seawall just E of the S end of the Golden Gate Bridge, San Francisco (moderately exposed),

PILLAR PT. (37°30′N, 122°30′W)—from a bedrock bench at the N end of Halfmoon Bay, approximately halfway from the base of the cliffs to the outermost rocks (exposed), and

HALFMOON BAY HARBOR (37°29′N, 122°24′W)—from rocks and debris along the otherwise sandy north shore of the harbor inside the breakwater (very protected).

These samples of 20 to 50 snails were frozen and sent to UCSD where they were stored at −70°C until electrophoresed. In preparation for electrophoresis, individuals were thawed, and the head-foot tissue was quickly separated from the digestive tissue and placed in Eppendorf tubes on ice. Chilled distilled water (0.1 mL) was added to the tubes and the tissues were homogenized with a glass rod. The homogenate was then centrifuged for 2 min at 10,000 *g*, and the supernatant absorbed onto 3 × 9 mm wicks of Whatman No. 3 chromatography paper. The wicks were blotted and loaded into chilled horizontal 12% starch (Sigma Chemical Co., St. Louis, Missouri) gels. The electrophoretic procedures followed those of WOODRUFF (1975) and MULVEY & VRIJENHOEK (1981). The particular buffer used for each of the 11 examined enzyme systems depended upon the enzyme (Table 1). At least two samples were included on each gel, the arrangement of which was varied to permit informative comparisons about the relative mobilities of alleles from different populations. Isozymes were assigned Roman numerals, and allozymes assigned values corresponding to their relative mobility (relative to the common allele in Bamfield) in order of decreasing mobility toward the anode.

The electrophoretic data were analyzed using BIOSYS-1 (SWOFFORD & SELANDER, 1981). Chi-square and Fisher exact tests for Hardy-Weinberg equilibrium were employed to examine the assumption of panmixia. The genetic structure of the population was examined using WRIGHT's (1978) hierarchical *F*-statistics. NEI's (1978) unbiased genetic distance and ROGERS' (1972) genetic similarity were calculated and clustered using the UPGMA algorithm.

Table 1

Buffers used to resolve protein variation in *Nucella emarginata*.

Enzyme (EC No.)	Abbreviation	Buffer†
Aspartate aminotransferase (2.6.1.1)	*Aat*	TBE 8.0
Esterase (1.11.1.6)	*Est*	LiOH
Glucose phosphate isomerase (5.3.1.9)	*Gpi*	TBE 8.0
Glucose-6-phosphate dehydrogenase (1.1.1.49)	*G6pd*	TC 6.8
Isocitrate dehydrogenase (1.1.1.42)	*Idh*	TC 6.8
Malate dehydrogenase (1.1.1.37)	*Mdh*	TC 6.8
Malic enzyme (1.1.1.40)	*Me*	TBE 8.0
Peptidase$_{la}$ (3.4.11)	*Pep$_{la}$*	LiOH
Peptidase$_{lgg}$ (3.4.11)	*Pep$_{lgg}$*	TBE 9/8
Phosphoglucomutase (2.7.5.1)	*Pgm*	TBE 9/8
Sorbitol dehydrogenase (1.1.14)	*Sordh*	LiOH

† TC 6.8: 0.188 M Tris, 0.065 M citrate, pH 6.8; diluted 1:10 for gels and 1:5 for electrodes (running time 18–21 hr at 150 V). TBE 8.0: 0.5 M Tris, 0.065 M borate, 0.02 M EDTA, pH 8.0; undiluted (18–21 hr, 80–100 V). TBE 9/8: 0.087 M Tris, 0.086 M borate, 0.001 M EDTA, pH 9.0; diluted 1:3 for gels. 0.5 M Tris, 0.065 M borate, 0.02 M EDTA, pH 8.0; undiluted for electrodes (17–21 hr, 80 V). LiOH: Solution A—0.03 M LiOH, 0.19 M borate, pH 8.1; Solution B—0.008 M citrate, 0.05 M Tris, pH 8.4; 10% A plus 90% B for gel, undiluted A for electrodes (18–22 hr, 120–150 V).

RESULTS

Within- and Between-Population Crosses

Except for the occasional cross, all within-population crosses produced at least one clutch, and most yielded several (Table 2a). In only 3 of these 36 crosses did capsules contain infertile eggs, and each was initiated with snails from a different source population. The average percentage of clutches that hatched ranged from 89 to 100% among the four source populations (Figure 1a).

Between-population crosses involving one parent from California yielded evidence of reproductive isolation. Only two of 19 between-population crosses failed to produce any egg capsules at all, and both were BC × WA crosses initiated with mature females (Table 2b). Of those between-population crosses that did produce egg capsules, however, with one exception, the only crosses that failed to yield viable embryos involved one parent from California (crosses 82-32 to 82-38, Table 2b; Figure 1b). The one exception was an AK × BC cross (80-12), but all the clutches of the remaining seven AK × BC crosses developed normally.

When surviving parents of crosses involving one California snail (82-32 to 82-38) were re-paired with those from their native population, all produced additional capsules (Table 2c). Significantly, all of these crosses, which included parents from Alaska, British Columbia, and California, yielded egg capsules with fertile embryos (Figure 1c). Furthermore, in all but one instance, 100% of the clutches were viable (Table 2c).

Variation in Egg-Capsule Morphology and Shell Sculpture

Snails from the northern populations used in the hybridization experiments produced egg capsules whose shape differed from those of the California population. Typically, capsules produced by snails from Alaska and British Columbia were more vase shaped with a proportionally lon-

Table 2

Outcome of crosses within and among four widely separated populations of *Nucella emarginata* along the Pacific Coast of North America. AK = Torch Bay, Alaska, BC = Bamfield, British Columbia, CA = Santa Barbara, California, WA = San Juan Island, Washington. See methods and Figure 4 for exact locations. Dates are Month/Day/Year. Date of first clutch refers to the date on which egg capsules were first noted.

Group	Cross label	Parents				Date cross initiated (m/d/y)	Time to first clutch (mo)	Duration of cross (mo)	Clutches		Ave. no. capsules per clutch	Ave. no. clutches per mo of laying
		Male		Female								
		Source	Size (mm)	Source	Size (mm)				*n*	% hatched		
a) Original within-population crosses												
AK	80-15	AK	12.4	AK	12.4	9/2/80	7.0§	16.1	3	100	14.7 ± 9.45	0.33
	80-16	AK	12.0	AK	14.2	9/2/80	6.0§	16.1	3	100	16.7 ± 0.58	0.30
	82-55	AK	19.8	AK	16.9	9/6/82	7.0	20.3	6	100	8.7 ± 4.89	0.45
	82-56	AK	19.8	AK	18.1	9/6/82	7.0	16.2	2	100	14.5 ± 9.19	0.22
	82-57	AK	19.6	AK	20.3	9/6/82	6.3	16.2	4	100	8.5 ± 3.79	0.40
	82-58	AK	21.2	AK	19.8	9/6/82	6.5	25.6	3	100	13.3 ± 2.08	0.16
	82-59	AK	20.2	AK	19.8	9/6/82	6.1	17.8	3	100	12.7 ± 3.21	0.26
	82-60	AK	16.3	AK	20.2	9/6/82	7.0	16.2	1	100	5 —	0.11
	82-61	AK	16.5	AK	18.8	9/6/82	5.9	17.8	5	100	11.6 ± 5.18	0.42
	82-62	AK	17.8	AK	23.0	9/6/82	5.9	20.8	6	100	13.3 ± 3.01	0.40
	82-63	AK	19.0	AK	15.2	9/6/82	13.4	24.5	1	?	2 —	0.09
	82-64	AK	19.4	AK	18.3	9/6/82	19.7	30.0	2	100	8.5 ± 2.12	0.19
	82-65	AK	22.6	AK	20.3	9/6/82	7.0	20.3	2	100	6.5 ± 0.71	0.15
	82-66	AK	18.8	AK	21.8	9/6/82	9.6	33.9	5	80	8.8 ± 3.42	0.21
	82-67	AK	19.8	AK	21.5	9/6/82	7.0	17.8	3	100	14.7 ± 6.03	0.28
	82-68	AK	15.8	AK	19.8	9/6/82	6.1	17.8	3	100	9.7 ± 2.89	0.25
	82-69	AK	19.2	AK	16.2	9/6/82	7.7	24.5	2	100	9.0 ± 1.41	0.12
	82-70	AK	20.3	AK	20.1	9/6/82	7.5	17.1	2	100	12.5 ± 6.36	0.21
BC	80-17	BC	14.0	BC	13.6	9/2/80	3.0§	16.1	6	100	16.3 ± 6.65	0.46
	80-18	BC	12.3	BC	12.2	9/2/80	3.0§	16.1	8	100	13.1 ± 5.28	0.61
	82-25	BC	14.6	BC	18.0	2/24/82	7.3†	14.9	4	100	12.5 ± 3.11	0.53
	82-26	BC	16.8	BC	16.0	5/9/82	7.3	16.2	9	100	8.4 ± 4.22	1.00
	82-27	BC	15.4	BC	18.5	1/1/82	9.1†	20.5	6	100	12.2 ± 3.19	0.53
	82-28	BC	16.3	BC	19.5	1/1/82	6.4†	20.5	10	100	13.4 ± 3.37	0.71
	82-51	BC	17.5	BC	13.2	6/15/82	6.0	14.7	7	0	12.6 ± 4.08	0.81
	82-52	BC	12.2	BC	16.0	6/15/82	7.7	19.0	5	100	10.6 ± 2.70	0.44
	82-53	BC	12.3	BC	12.7	6/15/82	8.6	19.0	3	100	8.0 ± 1.00	0.29
WA	82-40	WA	18.0	WA	18.4	6/10/82	5.1	15.2	6	100	13.2 ± 4.67	0.60
	82-41	WA	18.6	WA	27.9*	6/10/82	7.6	14.9	3	0	12.3 ± 3.21	0.41
	82-42	WA	15.6	WA	17.2	6/10/82	5.8	20.8	3	100	17.7 ± 14.0	0.20
	82-43	WA	18.0	WA	16.9	6/10/82	9.9	22.6	4	75	12.3 ± 6.70	0.31
	82-44	WA	14.9	WA	20.1	6/10/82	8.8	20.8	1	100	12 —	0.08
	82-45	WA	16.6	WA	17.7	6/10/82	6.2	19.1	6	100	15.7 ± 7.12	0.46
	82-46	WA	21.8*	WA	19.6	6/10/82	6.2	15.2	5	100	12.8 ± 5.26	0.56
CA	82-30	CA	21.9*	CA	19.9*	2/25/82	9.7	18.7	8	100	12.8 ± 2.49	0.89
	82-31	CA	18.4*	CA	25.3*	2/25/82	7.5†	17.0	3	100	14.7 ± 3.79	0.31

Table 2

Continued.

Group	Cross label	Parents: Male Source	Parents: Male Size (mm)	Parents: Female Source	Parents: Female Size (mm)	Date cross initiated (m/d/y)	Time to first clutch (mo)	Duration of cross (mo)	Clutches: *n*	Clutches: % hatched	Ave. no. capsules per clutch	Ave. no. clutches per mo of laying
b) Original between-population crosses												
AK × BC	80-01	AK	12.9	BC	13.7	9/2/80	3.0§	21.7	9	100	16.2 ± 6.42	0.48
	80-02	AK	12.3	BC	15.2	9/2/80	3.0§	21.7	9	100	17.9 ± 8.92	0.48
	80-03	BC	14.1	AK	16.3	9/2/80	3.0§	16.1	5	100	11.8 ± 6.69	0.38
	80-04	BC	14.5	AK	14.2	9/2/80	7.0§	21.7	5	100	14.2 ± 7.50	0.34
	80-11	AK	11.9	BC	11.7	9/2/80	3.0§	21.7	10	100	15.4 ± 4.45	0.54
	80-12	AK	13.7	BC	14.0	9/2/80	3.0§	21.7	6	0	16.0 ± 6.00	0.32
	80-13	BC	14.0	AK	15.0	9/2/80	7.0§	16.1	2	100	26.0 ± 2.83	0.22
	80-14	BC	12.2	AK	13.5	9/2/80	7.0§	21.7	3	100	12.3 ± 5.86	0.21
AK × CA	82-32	CA	19.9*	AK	24.0	2/25/82	13.9	18.7	2	0	9.0 ± 8.49	0.42
	82-33	CA	21.2*	AK	19.6	2/25/82	13.4	18.7	2	0	16.0 ± 11.3	0.38
	82-34	CA	16.1	AK	13.9	2/25/82	14.4	18.7	2	0	5.5 ± 2.12	0.47
	82-35	AK	23.2	CA	12.5	2/25/82	—	18.7	0	—	—	—
BC × WA	82-47	BC	20.8	WA	26.3*	6/10/82	2.0	15.2	8	88	13.8 ± 6.80	0.61
	82-48	BC	19.2	WA	26.8*	6/10/82	—	11.6	0	—	—	—
	82-49	BC	22.9*	WA	26.1*	6/10/82	1.3	15.2	9	78	13.1 ± 5.01	0.65
	82-50	BC	19.2	WA	28.5*	6/10/82	—	11.6	0	—	—	—
BC × CA	82-36	CA	21.5*	BC	14.5	2/25/82	7.5†	18.7	10	0	12.4 ± 6.33	0.90
	82-37	CA	20.4*	BC	14.7	2/25/82	7.5†	18.7	6	0	13.8 ± 7.00	0.54
	82-38	BC	17.7	CA	19.0*	2/25/82	7.5†	18.7	11	0	14.1 ± 4.32	0.99
c) Fertility tests of snails used in between-population crosses involving California parents												
AK	83-22	82-35‡	29.8*	82-32	28.8*	9/8/83	2.5	18.4	3	100	8.7 ± 9.07	0.19
	83-23	AK	28.8*	82-33	27.0*	9/8/83	8.1	13.3	2	100	9.0 ± 4.24	0.38
	83-24	AK	28.1*	82-34	27.8*	9/8/83	6.9	22.6	4	25	5.0 ± 3.16	0.25
BC	83-20	BC	14.2	82-36	28.5*	9/8/83	3.0	13.9	6	100	8.8 ± 6.77	0.55
	83-21	82-38	27.7*	82-37	29.1*	9/8/83	3.7	13.6	7	100	9.6 ± 5.09	0.70
CA	83-25	82-37	25.1*	82-38	27.9*	9/8/83	2.8	17.8	8	100	12.9 ± 5.87	0.53
	83-26	82-33	24.2*	CA	15.2	9/8/83	—	6.8	0	—	—	—
	83-27	82-34	26.4*	CA	18.1	9/8/83	8.6	22.2	3	100	13.3 ± 9.61	0.22
	83-28	82-36	30.6*	CA	16.6	9/8/83	—	8.7	0	—	—	—

* Mature parent.
§ Estimated from apparent age of capsules.
† Estimated ± 30 days.
‡ Number of between-population cross in which parent was originally used.

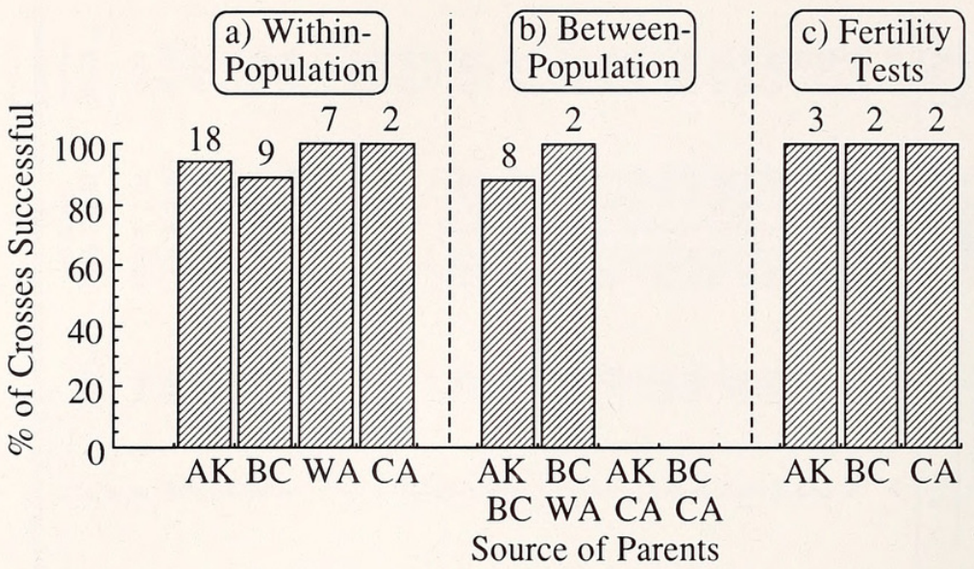


Figure 1

Percent of within- and between-population crosses with *Nucella emarginata* that were successful (*i.e.,* yielded at least some egg capsules with viable embryos; see Table 2) for parents originating from four different populations. Origins of parents: AK—Torch Bay, Alaska; BC—Bamfield, British Columbia; WA—San Juan Island, Washington; CA—Santa Barbara, California (see methods and Figure 4 for exact locations). Numbers above bars equal number of crosses; $n = 3$ for AK × CA and BC × CA.

ger neck (Figure 2a, b) compared to those from the California population. Capsules from the California parents were much more cylindrical and had a proportionally shorter neck that was often flared distally (Figure 2d). These differences in egg-capsule morphology were also used to identify parapatric populations of the putative "northern" and "southern" forms (Figure 2c and 2e, respectively) to test for gene flow using protein electrophoresis.

In addition, the form of spiral sculpture differed consistently between populations north and south of the San Francisco Bay area. Where sculpture was well developed, northern populations produced spirally uniform ribs (Figures 3, 4), although crenulations and growth checks sometimes caused minor, irregular variations in rib height. In southern populations, however, where sculpture was prominent it always included regularly spaced knobs along the distal margin of the spiral ribs (Figures 3, 4). Unfortunately, no consistent differences in shell shape were observed between populations north and south of the San Francisco Bay area. Therefore, in populations from wave-exposed shores where spiral sculpture was very weakly developed or absent, shells could not be identified reliably as "northern" or "southern."

Allozyme Variation

Consistent and genetically interpretable results were obtained for 18 loci from all samples (Tables 3, 4). In all six populations the mean number of alleles per locus (A) ranged from 1.1 to 1.2. The percentage of polymorphic loci (P) varied from 10.5 to 21.1% (loci were considered polymorphic if more than one allele was detected). Mean individual heterozygosity (H; by direct count) ranged from 0.009 to 0.041.

Only a few samples appeared to depart from panmixia. Among the 23 chi-square tests involving polymorphic loci (Table 3), 20 met Hardy-Weinberg expectations ($p > 0.05$). Only one locus at each of three sites did not: the *Aat-1* locus from Bamfield ($p = 0.033$), the *Lap-1* locus from Bodega Head ($p = 0.004$), and the *Lap-1* locus from Bodega Harbor ($p = 0.009$). Three significant results out of 23 independent chi-square tests were only slightly higher than that expected due to chance. Wright's F-statistics for all loci revealed a departure from panmixia ($F_{IS} = 0.188$), largely due to variation at the *Aat-1, Lap-1,* and *Pgm-2* loci. We are presently unable to account for these results as our sampling was not designed to address such questions. Pooling of different year classes may have contributed to this heterogeneity.

Cluster analysis of multilocus genetic-distance measures between the different populations (Table 5, Figure 5) revealed that the Bodega Head and Bodega Harbor populations were nearly identical, as were those from Pillar Pt. and Fort Pt. These four California populations were also closely related to each other with a genetic distance of $D = 0.01$, and were all moderately differentiated from the Bamfield population with a mean distance of $D = 0.11$. In contrast, the five northern populations differed considerably from the Halfmoon Bay population, with a mean genetic distance of $D = 0.20$ (Figure 5).

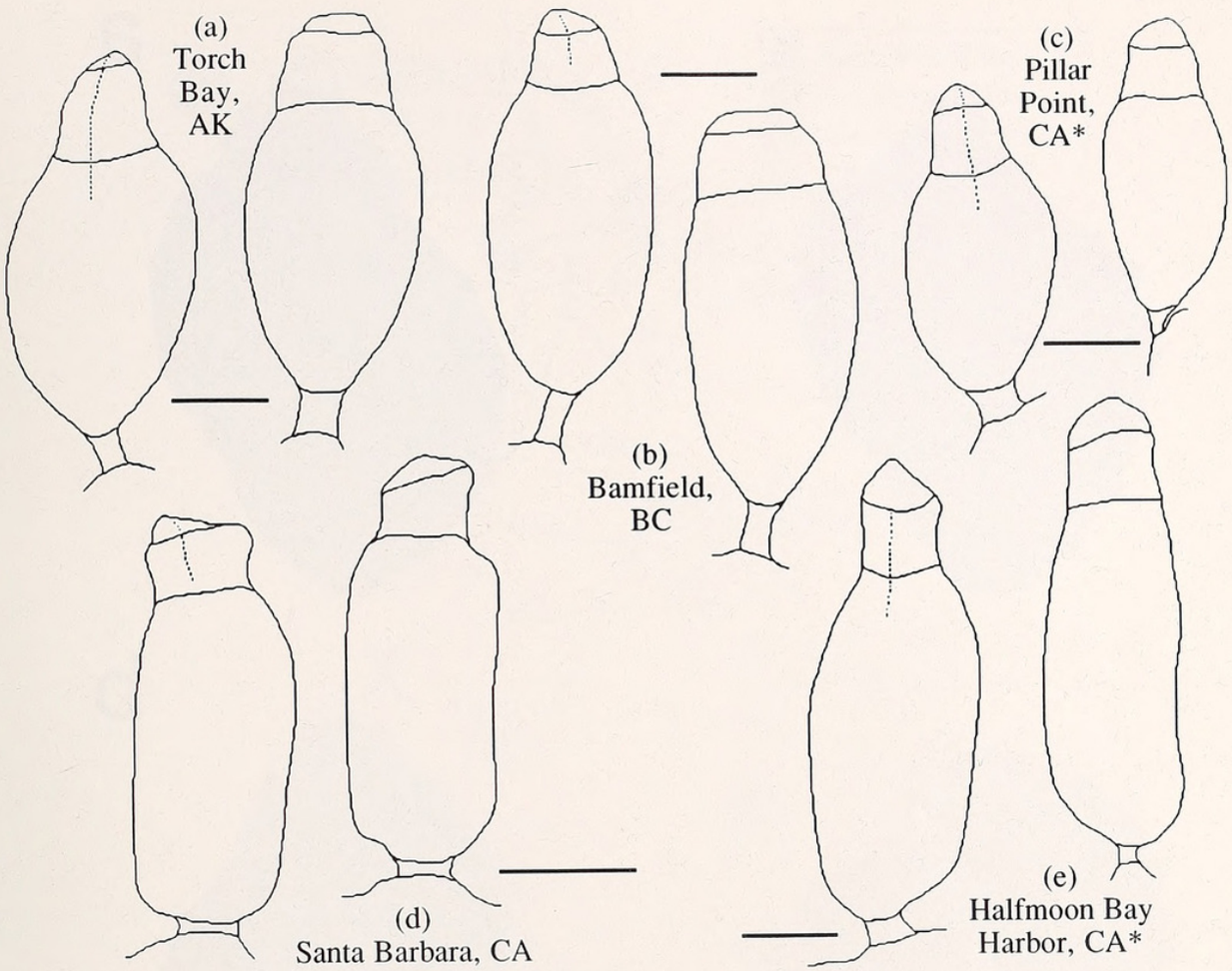


Figure 2

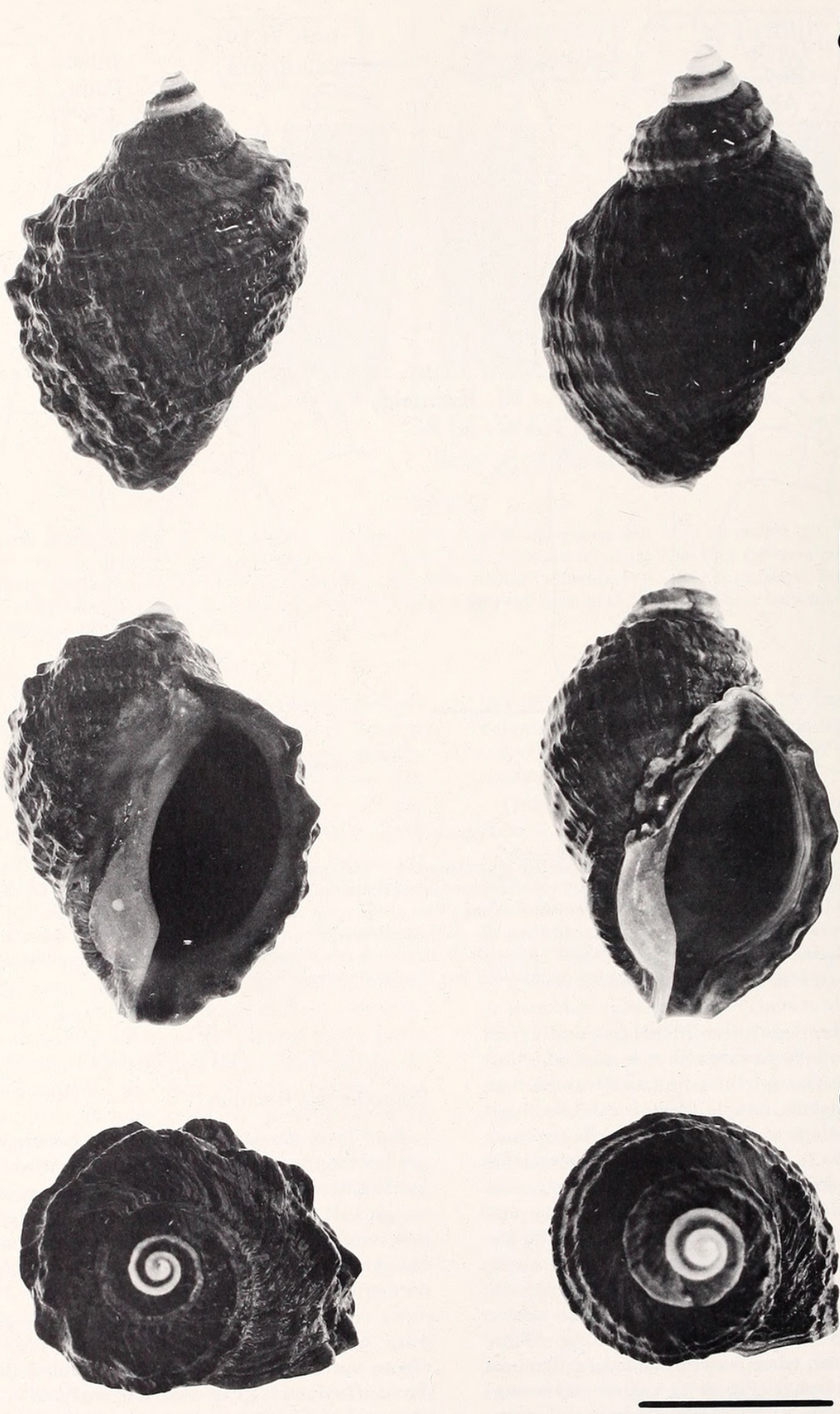
Camera lucida drawings of egg capsules from five populations of *Nucella emarginata* (see methods and Figure 4 for exact locations). Two views of an individual, representative capsule are illustrated for each population: a seam view (dotted line along neck) and a view perpendicular to the plane of the seam. Capsules (a), (b), and (d) were laid in the laboratory and (c) and (e) were collected from the field. Capsules (a)–(c) illustrate the "northern" form and capsules (d) and (e) illustrate the "southern" form. Scale bars = 2 mm (capsules not all drawn to same scale). *Note that (c) and (e) were collected from populations less than 500 m from each other.

The Halfmoon Bay population differed genetically from the remaining populations examined in several other important respects, even though this population was less than 500 m from the Pillar Pt. sample. First, it exhibited fixed allelic differences at one of the eight loci exhibiting more than one allele (*Idh-2*). Second, at two of the remaining seven loci (*Aat-1* and Pep_{la}-*2*), a unique allele was observed at moderate to high frequency in the Halfmoon Bay population. Third, even with allozyme data included for the population from Bamfield, BC, some 1600 km to the north, the Halfmoon Bay population was the most genetically distinct of all those examined (Figure 5). These genetic differences between the Halfmoon Bay and the nearby Pillar Pt. populations, when coupled with the differences in egg-capsule morphology (Figure 2), suggest very strongly an absence of gene flow between the northern and southern cryptic species.

DISCUSSION

Reproductive Isolation

Four lines of evidence suggest that complete reproductive isolation has evolved between central Californian and northeastern Pacific populations of the species currently recognized as *Nucella emarginata*. First, the only crosses that consistently yielded egg capsules whose embryos did not develop were hybrid crosses between Californian and northern populations (Table 2b, Figure 1b). The infertile capsules produced by these crosses were important evidence because they showed that neither laboratory conditions nor lack of a suitable mate inhibited capsule production. Inviable egg capsules were produced in a few other crosses (3 of 37 within-population [Table 2a], and 1 of 10 between-population crosses [Table 2b]), but no more than


a
b
c

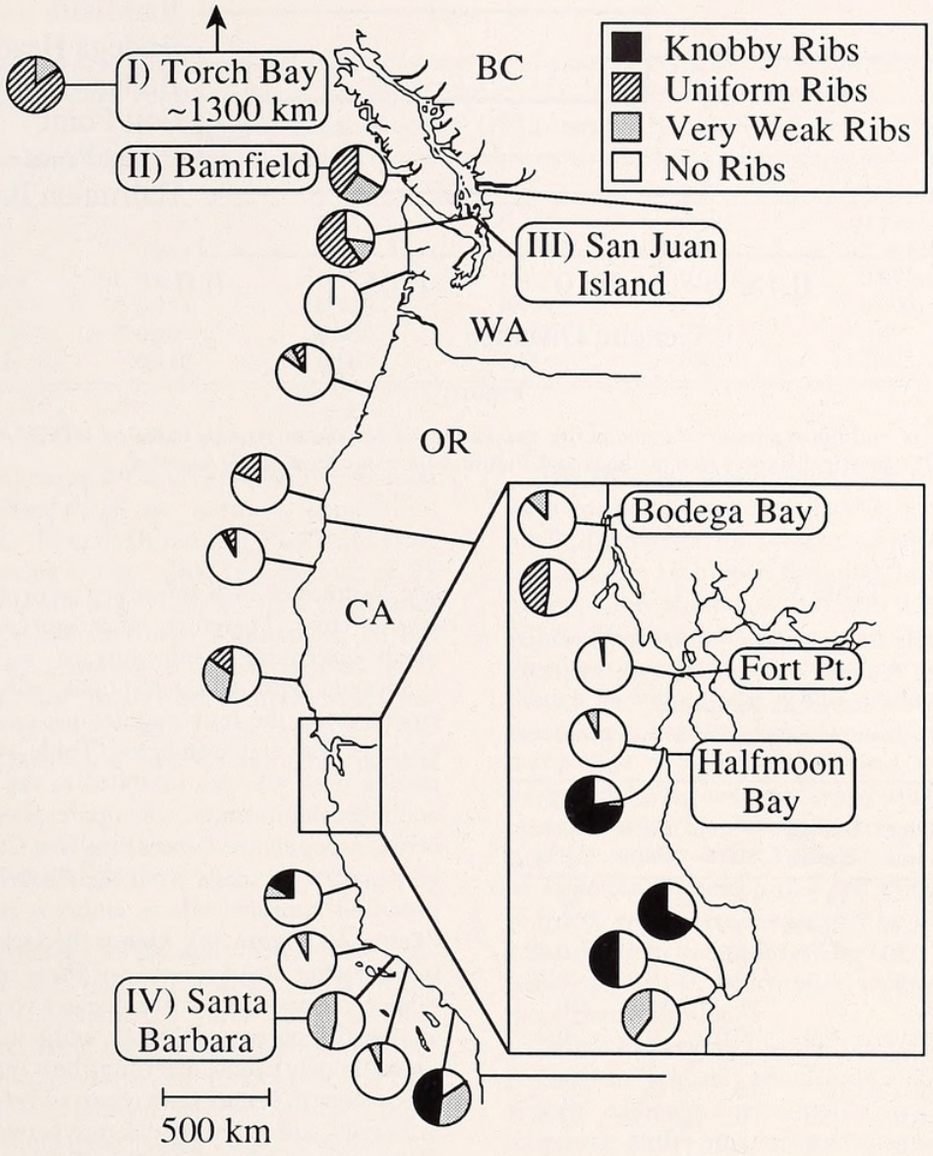


Figure 4

Frequencies of different forms of spiral sculpture in populations of *Nucella emarginata* from the west coast of North America. Sites preceded by Roman numerals indicate populations used to assess reproductive isolation (see Table 2 and Figure 1). Solid sections—regularly spaced knobs present on spirally sculptured individuals; cross-hatched sections—no regularly spaced knobs evident on spirally sculptured individuals; stippled sections—sculpture present but too weak to identify as uniform or knobby; open sections—smooth-shelled individuals. Sample sizes ranged from 13 to 191 (median n = 37).

←

Figure 3

Forms of spiral shell sculpture for the southern form (left, Halfmoon Bay), and northern form (right, Grappler Inlet, Vancouver Island, British Columbia, 48°49′54″N, 125°07′00″W) of *Nucella emarginata*. Note the prominent, regularly spaced knobs along the ribs of the southern form. a, abapertural view; b, apertural view; and c, apical view. These shells, along with others from the same site, have been deposited in the Los Angeles County Museum (accession numbers 86-449.1 and 86-448.2 for the southern and northern forms, respectively). Scale bar = 10 mm. Egg capsules for the "northern" and "southern" species have been similarly deposited (accession numbers 86-447.1 and 86-448.1, for Bodega Bay Harbor and Halfmoon Bay Harbor respectively).

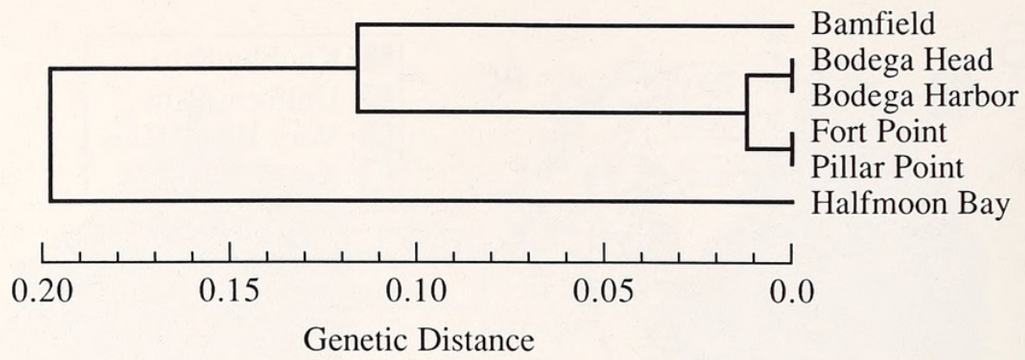


Figure 5

Phenetic tree of multilocus similarities among five populations of *Nucella emarginata* based on UPGMA clustering of NEI's (1978) genetic distances (see methods and Figure 4 for exact locations of samples).

Table 3

Sample sizes, and allele frequencies of allozyme variants, for six populations of *Nucella emarginata*† (see methods and Figure 4 for exact locations).

Locus/ Allele RM*	Population					
	Bamfield	Bodega Head	Bodega Harbor	Fort Point	Pillar Point	Halfmoon Bay
Aat-1	25§	11	14	14	15	29
100	0.62	0.50	0.50	0.07	0.03	0.0
86	0.38	0.50	0.50	0.93	0.97	0.62
71	0.0	0.0	0.0	0.0	0.0	0.38
Idh-1	40	20	20	15	15	30
106	0.0	0.03	0.0	0.0	0.0	0.0
100	1.0	0.97	1.0	1.0	1.0	1.0
Idh-2	40	20	18	15	15	30
109	0.0	0.0	0.0	0.0	0.0	1.0
100	1.0	1.0	1.0	1.0	1.0	0.0
Lap-1	31	15	17	4	10	23
100	1.0	0.33	0.32	0.13	0.20	0.02
90	0.0	0.67	0.68	0.87	0.80	0.98
Pep_{la}-*2*	40	20	20	15	15	30
100	1.0	0.03	0.03	0.0	0.0	0.0
85	0.0	0.97	0.97	1.0	1.0	0.05
38	0.0	0.0	0.0	0.0	0.0	0.95
Pep_{lgg}	40	15	15	10	10	30
100	0.60	0.97	0.87	0.90	0.85	0.02
97	0.0	0.03	0.13	0.10	0.15	0.98
95	0.40	0.0	0.0	0.0	0.0	0.0
Pgm-2	30	20	20	15	15	20
105	0.17	0.0	0.0	0.0	0.0	0.0
100	0.83	1.0	1.0	1.0	1.0	1.0
Sordh	35	10	10	10	10	25
100	0.97	1.0	1.0	1.0	1.0	1.0
82	0.03	0.0	0.0	0.0	0.0	0.0

† Monomorphic loci: *Aat-2, Est, Gpi, Lap-2, Ldh, Mdh-1, Mdh-2, Me,* Pep_{la}*-1, Pgm-1.*

* RM: relative mobility, decreasing toward anode.

§ Number of individuals.

one instance of such infertility occurred within any category of cross. Therefore, no category of cross was particularly susceptible to infertility.

Second, with few exceptions, all within-population crosses from the four populations examined yielded egg capsules with viable embryos (Table 2a, Figure 1a); hence mating itself was not inhibited in the laboratory. Third, and most significantly, when parents originally present in between-population crosses involving California snails were re-paired with snails from their source population, they produced capsules whose embryos developed normally (Table 2c, Figure 1c). Hence the lack of fertile capsules in the rather small number of these initial between-population crosses was not just due to a chance lack of fertility among the parents. Without additional information, we unfortunately cannot determine how reproductive isolation was achieved. It may have occurred before or after mating.

Finally, although the distance between Santa Barbara, California, and Bamfield, British Columbia (ca. 1800 km) is comparable to that between Bamfield and Torch Bay,

Table 4

Mean sample size per locus (n), mean number of alleles per locus (A), percentage of loci polymorphic (P), and average number of heterozygous loci per individual (H, by direct count) for six populations of *Nucella emarginata* analyzed for allozyme variation (computed from data in Table 3).

Population	n	A	P	H
Bamfield	36.6	1.2	21.1	0.041
Bodega Head	18.4	1.2	21.1	0.017
Bodega Harbor	18.4	1.2	15.8	0.022
Fort Pt.	13.4	1.1	10.5	0.024
Pillar Pt.	13.7	1.2	10.5	0.037
Halfmoon Bay	25.9	1.2	15.8	0.009

Table 5

Genetic similarity and genetic distance estimates among six populations of *Nucella emarginata* from the Pacific Coast of North America. Above diagonal: NEI's (1978) unbiased genetic distance. Below diagonal: ROGERS' (1972) genetic similarity.

Population	1	2	3	4	5	6
1 Bamfield	—	0.145	0.148	0.187	0.182	0.313
2 Bodega Head	0.828	—	0.000	0.010	0.012	0.184
3 Bodega Harbor	0.829	0.992	—	0.010	0.011	0.173
4 Fort Pt.	0.794	0.959	0.964	—	0.000	0.164
5 Pillar Pt.	0.797	0.958	0.967	0.991	—	0.162
6 Halfmoon Bay	0.712	0.807	0.814	0.827	0.825	—

Alaska (ca. 1300 km), no evidence of reproductive isolation was apparent between these two northern populations (Table 2b; PALMER, 1984a). All but one F1 hybrid cross yielded viable offspring (Table 2b). In addition, of 28 crosses initiated with offspring from all six hybrid lines between these two distant northern populations, all but one yielded viable F2 offspring: 80-1 (six crosses), 80-2 (eight crosses), 80-3 (four crosses), 80-4 (three crosses, one of which yielded no capsules), 80-11 (one cross), and 80-13 (six crosses). Thus, these hybrids exhibited normal fertility.

Isolation by Distance or Two Discrete Species?

Because of the distance (≥1800 km) between the Californian (Santa Barbara) population and those from the northeastern Pacific (Bamfield and Torch Bay) examined in this study, and because of the low gene flow presumably associated with direct development (GRANT & UTTER, 1988), the reproductive isolation observed between these distant populations could reflect either isolation by distance (WRIGHT, 1943), where all adjacent populations along the coast could interbreed, or the presence of two discrete species. Taken together, however, the concordant patterns of variation in the form of spiral shell sculpture, egg-capsule morphology, and in allozymes all suggest the presence of two discrete species.

Shell sculpture also varied in a manner consistent with two discrete species. Among shells that exhibited spiral sculpture, regularly spaced knobs along the distal margin of the ribs only occurred south of San Francisco Bay (Figure 4). The absence of this form of sculpture north of San Francisco Bay suggested that if two species did exist, the southern one did not extend north of this area. This pattern alone, however, also could have been produced by a genetically based sculpture polymorphism where the allele for "knobby ribs" had not yet crossed the mouth of San Francisco Bay. Evidence for Mendelian inheritance of shell sculpture already exists for Vancouver Island populations of *Nucella emarginata* (PALMER, 1985b).

The allozyme data provide the most convincing evidence for two discrete species (Tables 3, 5, Figure 5). Fixed allelic differences, the presence of novel alleles at moderate frequency in Halfmoon Bay, and the high genetic distance between the Halfmoon Bay and Pillar Pt. populations, which were less than 500 m apart, all suggest that no gene flow was occurring between these two populations. Although no simple relationship exists between genetic distance and taxonomic level, the discovery of a genetic distance of $D = 0.16$ between these adjacent samples was unexpected for conspecific populations. Not only were the four remaining San Francisco area samples virtually identical ($D = 0.00$–0.01; Table 5), but the genetic distance between the Halfmoon Bay and Pillar Pt. populations ($D = 0.16$), which were separated by less than 500 m, was comparable to that observed between Pillar Pt. and Bamfield ($D = 0.18$), which were separated by a distance of more than 1300 km.

Before proceeding further, remember that allozyme differences are probably not directly involved in the speciation process. Although a relationship may exist between degree of genetic differentiation and taxonomic status in some groups of mollusks, a quantitative relationship is not predictable from first principles. Nonetheless, we note that in a survey of over 7000 comparisons of conspecific populations, only 2% of the intraspecific D estimates exceeded 0.10 (THORPE, 1983). A review of published intraspecific and interspecific genetic distances in mollusks supports our interpretation of the separate species-level status of the Halfmoon Bay population (STAUB *et al.*, 1990; WOODRUFF *et al.*, 1988).

Consistent with the allozyme differences, the egg capsules produced by snails from Pillar Pt. were of the "northern" form while those from Halfmoon Bay snails were "southern" in appearance (Figure 2c and e, respectively). Therefore, although the Pillar Pt. snails did not exhibit strong enough spiral sculpture to determine its form, differences in egg-capsule morphology between these two populations also support our interpretation of two discrete species.

Other ecological differences also exist between these two

putative species. Northern populations of *Nucella emarginata* feed almost exclusively upon balanomorph barnacles and mussels, and rarely on limpets (PALMER, 1980, 1988). Southern populations, on the other hand, feed commonly upon the gooseneck barnacle *Pollicipes* and consistently, although in low frequency, upon limpets (WEST, 1986). In addition, individuals in northern populations do not exhibit any evidence of "majoring" (HEINRICH, 1979) on particular prey types (PALMER, 1984b), while those in southern populations do (WEST, 1986).

Shell Form and Cryptic Species

The discovery of two morphologically very similar species within the current taxon *Nucella emarginata* highlights the difficulty of recognizing species based upon differences in shell form. Cryptic species have recently been identified by allozyme differences in several prosobranch genera including *Brotia* (KLINHOM, 1989), *Collisella* (MURPHY, 1978), *Crepidula* (HOAGLAND, 1984), *Goniobasis* (CHAMBERS, 1980), *Littorina* (MASTRO *et al.*, 1982; BOULDING, 1990), *Neotricula* (STAUB *et al.*, 1990), *Oncomelania* (WOODRUFF *et al.*, 1988), and *Robertsiella* (YONG *et al.*, 1985). Given the extent to which shells may vary within species, particularly in thaidine gastropods (CROTHERS, 1984; APPLETON & PALMER, 1988, and references therein), more cryptic species seem likely to be discovered.

Other Levels of Variation

Although chromosome differences are often associated with speciation (WHITE, 1978), a preliminary study of chromosome variation among northeastern Pacific *Nucella* revealed that all four species recognized at the time (*canaliculata, emarginata, lamellosa,* and *lima*) had the same haploid chromosome number (n = 35; AHMED & SPARKS, 1970). The constancy within and among these species suggests that the two cryptic species identified here will also lack differences in chromosome number.

This lack of chromosome variation in northeastern Pacific species is surprising in view of the striking polymorphism observed in the north Atlantic *Nucella lapillus*. In populations of *N. lapillus* from northern France, the haploid chromosome number varies more or less continuously from n = 18 to n = 13 over a wave-exposure gradient (STAIGER, 1954). In a much more detailed study, BANTOCK & COCKAYNE (1975) found this polymorphism to be limited to shores of southern England. More northern populations from the Bristol Channel and from the Straits of Dover were monomorphic for a haploid chromosome number of n = 13 (BANTOCK & COCKAYNE, 1975), as were both protected- and exposed-shore populations from the coast of Norway (HOXMARK, 1970). The larger scale correlation of chromosome number with latitude suggests that perhaps these chromosome forms reflect northern and southern races of *lapillus* that diverged in isolation and have since come back into contact. The common occurrence of intermediates in areas where both forms coexist (BANTOCK & COCKAYNE, 1975; STAIGER, 1954), however, indicates that hybrids are perfectly viable and that these chromosome differences are probably not associated with cryptic species.

Taxonomic Status of the New Species

Which of the two cryptic species identified above will retain the name *emarginata*? This question is not as straightforward as it might seem. In his second description, published in French, DESHAYES (1841) illustrated a shell whose form of spiral sculpture was ambiguous (spiral ribs were not clearly uniform or knobby). To exacerbate the situation, he cited the type locality as New Zealand! DALL (1915), without explaining the basis of his decision, reassigned the type locality to San Miguel Id., in the channel islands of California near Santa Barbara (see Figure 4). This new type locality would lie within the range of the putative southern taxon identified here.

To complicate matters further, generic relations within the Thaidinae are in a state of flux (KOOL, 1987). *Nucella* is the genus to which the northeast Pacific species previously called *Thais* are now referred (*e.g.*, KOZLOFF, 1987; MORRIS *et al.*, 1980; SMITH & CARLTON, 1975), and *Nucella* may in fact not be a true thaidine (KOOL, 1988). The evidence upon which this generic reassignment has been made is discussed at length in KOOL (1989).

Several synonyms and varietal names are available for *Nucella emarginata* (cited in DALL, 1915), including: *anomala* (Middendorff, 1849), *ostrina* (Gould 1852), *saxicola* (Carpenter, 1864), and *projecta* (Dall, 1915). *Nucella fuscata* [*Purpura*] (Forbes 1850; as cited in VANATTA, 1910) may also be eligible. We suggest that until a description and synonymy is completed, studies of "*Nucella emarginata*" should include a collection of egg capsules, and shells with well developed sculpture. Temporarily, at least, the species could be called *Nucella emarginata* (northern) and *Nucella emarginata* (southern).

ACKNOWLEDGMENTS

This research was funded by NSERC Operating Grant A7245 (to ARP) and, in part, by UCSD Academic Senate and NSF grants to DSW. The allozyme data were incorporated as part of a thesis (by SDG) submitted in partial fulfillment of the requirements for an MSc at UCSD. We thank Robin Boal for her dedication while maintaining the laboratory crosses, and the director and staff of the Bamfield Marine Station for their patience. Silvard Kool and two anonymous reviewers offered useful comments on the manuscript.

LITERATURE CITED

ABBOTT, R. T. 1974. American seashells. Van Nostrand Reinhold Co.: New York. 663 pp.

AHMED, M. & A. K. SPARKS. 1970. A note on the chromosome number and interrelationships in the marine gastropod genus *Thais* of the United States Pacific Coast. The Veliger 12: 293–294.

ANSELL, A. D. 1982. Experimental studies of a benthic predator-prey relationship. I. Feeding, growth, and egg collar production in long-term cultures of the gastropod drill *Polinices alderi* (Forbes) feeding on the bivalve *Tellina tenuis* (da Costa). Journal of Experimental Marine Biology and Ecology 56:235–255.

APPLETON, R. D. & A. R. PALMER. 1988. Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proceedings of the National Academy of Sciences (USA) 85:4387–4391.

BANTOCK, C. R. & W. C. COCKAYNE. 1975. Chromosomal polymorphism in *Nucella lapillus*. Heredity 34:231–245.

BOULDING, E. G. 1990. Systematics, ecology, and ecological genetics of some northeastern Pacific *Littorina*. Doctoral Dissertation, Zoology Department, University of Washington, Seattle. 239 pp.

CAMPBELL, C. A. 1978. Genetic divergence between populations of *Thais lamellosa* (Gmelin). Pp. 157–170. *In:* B. Battaglia & J. A. Beardmore (eds.), Marine organisms: genetics, ecology and evolution. Plenum Press: New York.

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

CROTHERS, J. H. 1984. Some observations on shell shape variation in Pacific *Nucella*. Biological Journal of the Linnean Society 21:259–281.

DALL, W. H. 1915. Notes on the species of the molluscan subgenus *Nucella* inhabiting the northwest coast of America and adjacent regions. Proceedings of the US National Museum 49:557–572.

DESHAYES, G. P. 1841. G. Poupre: *Purpura*. Magasin de Zoologie (Guerin), Pl. 25.

FRETTER, V. & A. GRAHAM. 1962. British prosobranch molluscs. Ray Society: London. 755 pp.

GRANT, W. S. & F. M. UTTER. 1988. Genetic heterogeneity on different geographic scales in *Nucella lamellosa* (Prosobranchia, Thaididae). Malacologia 28:275–288.

HEINRICH, B. 1979. "Majoring" and "minoring" by foraging bumblebees, *Bombus vagans*: an experimental analysis. Ecology 60:245–255.

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

HOXMARK, R. C. 1970. The chromosome dimorphism of *Nucella lapillus* (Prosobranchia) in relation to the wave exposure. Nytt Magasin for Zoologi 18:229–238.

KINCAID, T. 1964. Notes on *Thais* (*Nucella*) *lima* (Gmelin), a marine gastropod inhabiting areas in the North Pacific Ocean. Calliostoma Co.: Seattle. 41 pp.

KLINHOM, U. 1989. The Thiaridae (Prosobranchia: Gastropoda) of Thailand: their morphology, anatomy, allozymes and systematic relationships. Doctoral Dissertation, Mahidol University, Bankok.

KOOL, S. P. 1987. Significance of radular characters in reconstruction of thaidid phylogeny (Neogastropoda: Muricacea). The Nautilus 101:117–132.

KOOL, S. P. 1988. Aspects of the anatomy of *Plicopurpura patula* (Prosobranchia: Muricoidea: Thaidinae), new combination, with emphasis on the reproductive system. Malacologia 29: 373–382.

KOOL, S. P. 1989. Phylogenetic analysis of the subfamily Thaidinae (Prosobranchia: Neogastropoda: Muricidae). Doctoral Dissertation, George Washington University, Washington, D.C. 342 pp.

KOZLOFF, E. N. 1987. Marine invertebrates of the Pacific Northwest. University of Washington Press: Seattle. 511 pp.

MASTRO, E., V. CHOW & D. HEDGECOCK. 1982. *Littorina scutulata* and *Littorina plena*: sibling species status of two prosobranch gastropod species confirmed by electrophoresis. The Veliger 24:239–246.

MORRIS, R. H., D. P. ABBOTT & E. C. HADERLIE. 1980. Intertidal invertebrates of California. Stanford University Press: Stanford, California. 690 pp.

MULVEY, M. & R. C. VRIJENHOEK. 1981. Genetic variation among laboratory strains of the planorbid snail *Biomphalaria glabrata*. Biochemical Genetics 19:1169–1182.

MURPHY, P. G. 1978. *Collisella austrodigitalis* sp. nov.: a sibling species of limpet (Acmaeidae) discovered by electrophoresis. Biological Bulletin 155:193–206.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 7: 145–153.

PALMER, A. R. 1980. A comparative and experimental study of feeding and growth in thaidid gastropods. Doctoral Dissertation, Zoology Department, University of Washington, Seattle. 320 pp.

PALMER, A. R. 1984a. Species cohesiveness and genetic control of shell color and form in *Thais emarginata* (Prosobranchia, Muricacea): preliminary results. Malacologia 25:477–491.

PALMER, A. R. 1984b. Prey selection by thaidid gastropods: some observational and experimental field tests of foraging models. Oecologia 62:162–172.

PALMER, A. R. 1985a. Genetic basis of shell variation in *Thais emarginata* (Prosobranchia, Muricacea) I. Banding in populations from Vancouver Island. Biological Bulletin 169: 638–651.

PALMER, A. R. 1985b. Quantum changes in gastropod shell morphology need not reflect speciation. Evolution 39:699–705.

PALMER, A. R. 1988. Feeding biology of *Ocenebra lurida* (Prosobranchia: Muricacea): diet, predator-prey size relations, and attack behavior. The Veliger 31:192–203.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. University of Texas Publications in Genetics 7: 145–153.

SMITH, R. I. & J. T. CARLTON. 1975. Light's Manual. Intertidal invertebrates of the central California coast. 3rd ed. University of California Press: Berkeley. 717 pp.

STAIGER, H. 1954. Der Chromosomendimorphismus beim Prosobranchier *Purpura lapillus* in Beziehung zur Ökologie der Art. Chromosoma 6:419–478.

STAUB, K. C., D. S. WOODRUFF, E. S. UPATHAM, V. VIYANANT & H. C. YUAN. 1990. Genetic variation in *Neotricula aperta*, the intermediate host snail of *Schistosoma mekongi*: allozyme differences reveal a group of sibling species. American Malacological Bulletin 7:93–103.

STRATHMANN, M. F. 1987. Reproduction and development of marine invertebrates of the northern Pacific coast. University of Washington Press: Seattle. 670 pp.

SWOFFORD, D. L. & R. B. SELANDER. 1981. BIOSYS-1: a

FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281–283.

THORPE, J. P. 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. *In:* G. S. Oxford & D. Rollinson (eds.), Protein polymorphism: adaptive and taxonomic significance. Academic Press: London.

TRYON, G. 1880. Purpurinae. Manual of conchology. Vol. 2. 289 pp.

VANATTA, E. G. 1910. *Purpura crispata* and *saxicola*. The Nautilus 24:37–38,

VERMEIJ, G. J., A. R. PALMER & D. R. LINDBERG. 1990. Range limits and dispersal of mollusks in the Aleutian Islands, Alaska. The Veliger 33:346–354.

WEST, L. 1986. Interindividual variation in prey selection by the snail *Nucella* (=*Thais*) *emarginata*. Ecology 67:798–809.

WHITE, M. J. D. 1978. Modes of speciation. W. H. Freeman: San Francisco. 455 pp.

WOODRUFF, D. S. 1975. Natural history of *Cerion*. V. Allozyme variation and genic heterozygosity in the Bahamian pulmonate *Cerion bendalli*. Malacological Review 8:47–55.

WOODRUFF, D. S., K. C. STAUB, E. S. UPATHAM, V. VIYANANT & H. C. YUAN. 1988. Genetic variation in *Oncomelania hupensis*: *Schistosoma japonicum* transmitting snails in China and the Philippines are distinct species. Malacologia 29:347–361.

WRIGHT, S. 1943. Isolation by distance. Genetics 28:114–138.

WRIGHT, S. 1978. Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations. University of Chicago Press: Chicago. 580 pp.

YONG, H. S., C. S. OOI, G. J. GREER, K. P. F. LAI & C. K. OW-YANG. 1985. Biochemical genetic differentiation of three species of *Robertsiella* snails (Gastropoda: Prosobranchia: Pomatiopsidae), the intermediate host of a Malaysian schistosome. Tropical Biomedicine 2:113–120.