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Clonal and solitary anemones (*Anthopleura*) of western North America: population genetics and systematics

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Abstract

Francis (1979) proposed that clonal and solitary forms of the anemone *Anthopleura elegantissima* are actually two species. In 1984 and 1985, samples from two to six California populations of all known forms and species of Californian *Anthopleura* were analyzed electrophoretically to determine their taxonomic relationships. Data from 14 enzymes and 18 loci, 17 of them polymorphic, show that the two forms of *A. elegantissima* are virtually identical electrophoretically, and there is no evidence of reduced gene flow between them. We conclude there are three species of *Anthopleura* in California: *A. elegantissima* (Brandt, 1835), *A. xanthogrammica* (Brandt, 1835) and *A. artemisia* (Dana, 1848). Genetic variation in the two species capable of asexual reproduction, *A. elegantissima* and *A. artemisia,* is extremely high, approximately 2.5 times that of the strictly sexual *A. xanthogrammica.*

Introduction

The family Actiniidae is the largest known family of sea anemones (Cnidaria: Actinaria), containing at least 43 genera and 200 species (Carlgren, 1949). The genus *Anthopleura* includes at least 42 species, is widely distributed throughout the world (Carlgren, 1949; Hand, 1955; Dunn, 1977), and is most often found intertidally, especially in the upper littoral zone (Carlgren, 1949). Hand (1955) listed three species of *Anthopleura* from California, all living intertidally on the central coast: *A. xanthogrammica, A. elegantissima* and *A. artemisia.* While *A. xanthogrammica* had long been assigned to the genus *Anthopleura, A. elegantissima* previously had been assigned to the genus *Bunodactis,* while *A. artemisia* was included in the genus *Evactis.* No other species of *Anthopleura* are recognized in California, although *A. dowii* (Verrill, 1869) has been reported as far north as Mexico (Carlgren, 1951). In addition, the ranges of a number of western Pacific *Anthopleura* species may extend around the north Pacific and overlap the northern boundaries of the Californian species.

Hand (1955) based his recognition of three *Anthopleura* species upon morphological differences (including the abundance and distribution of verrucae on the columns) and on the structure and distribution of nematocysts within the anemones. Yet, despite the redescriptions by Hand (1955), field identification of the species is often difficult, due to the variability of external characteristics. The taxonomic situation within *Anthopleura* species is complicated further by the existence of two morphologically and ecologically distinct forms of *A. elegantissima* (Hand, 1955; Francis, 1973a, b). These are a small, clonal, aggregating anemone living in both high and low tidal zones and a large, solitary anemone found in more protected mid- to sub-tidal zones. The clonal form most often lives on open rock surfaces, where it is exposed to wave action and desiccation, while the solitary form is usually limited to more protected tidepools and crevices. The large, solitary form has often been confused with *A. xanthogrammica,* which it often resembles closely in size, morphology and habitat. As examples, Fig. 53 in Ricketts and Calvin (1968) and Fig. 16 in MacGinitie and MacGinitie (1968) both show solitary *A. elegantissima* mislabelled as *A. xanthogrammica.* Francis (1979) proposed that the two forms of *A. elegantissima* actually constitute a sibling species pair that can be distinguished by phenotypic frequencies (color markings), biogeographic ranges and microhabitat differences. Since a second species has not been described formally, the taxonomic status of *A. elegantissima* and its two ecological forms has been in question since the sibling species pair was proposed.

Differences in morphology, habit and reproduction of the four forms of *Anthopleura* are summarized in Table 1. *A. elegantissima* and *A. xanthogrammica* reproduce sexu-

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Table 1. *Anthopleura* spp. Distinguishing traits of Californian forms after Hand (1955), Francis (1973a, 1979)

Character	*A. elegantissima* clonal	*A. elegantissima* solitary	*A. xanthogrammica*	*A. artemisia*
Column diameter	small (< 6 cm)	large (4–12 cm)	large (4–12 cm)	small (< 5 cm)
Habit	clonal	solitary	solitary	solitary
Reproductive mode	sexual and asexual	sexual	sexual	sexual and asexual
Column verrucae	longitudinal rows of simple verrucae		numerous compound verrucae	simple, upper third of column
Disc color	varied with visible radiating mesenteric insertions		solid green or blue	varied with complex patterns

ally by external fertilization, producing pelagic, planktotrophic larvae (Ford, 1964; Siebert, 1974; Jennison, 1979). In addition, the small form of *A. elegantissima* reproduces asexually by longitudinal fission to create clonal aggregations (Ford, 1964; Francis, 1973a, b). On the basis of mesenteric scars, Hand (1955) inferred that *A. artemisia* also may reproduce asexually by longitudinal fission (Hand, 1955; Morris *et al.*, 1980). The mode of sexual reproduction in *A. artemisia* has not been described. Asexual reproduction is not known in *A. xanthogrammica*, nor in the large solitary form of *A. elegantissima*.

The known geographic ranges of the four forms of *Anthopleura* are summarized in Fig. 1A. The northern boundary of the solitary form of *A. elegantissima* and the southern boundary of *A. artemisia* occur in California, but the boundaries outside California are not well known. The continuous range of *A. xanthogrammica* ends at Pt. Conception, but it occurs further south in regions of cold upwellings (Francis, 1979; V. B. Pearse, personal communication), perhaps as far as Panama (Ricketts and Calvin, 1978; Morris *et al.*, 1980). The ranges of all four forms of Californian *Anthopleura* overlap in the California Transition Zone, a well documented region of rapid faunal change between northern and southern biotas (Valentine, 1966; Newman, 1979).

In this paper, we use electrophoretic allozyme data to characterize all forms of the genus *Anthopleura* found along the Californian coast with the primary objective of testing the suggestion of Francis (1979) that *A. elegantissima* includes two species. In the text, we refer to four "forms" of anemones until the taxonomic status of each has been determined. We also examine the electrophoretic data for intra-populational, inter-populational and interspecific patterns of genetic variability.

Materials and methods

Sites

In December, 1984 and March, 1985 samples were collected at five sites in the Monterey Bay area in central California where the distributions of the four forms of *Anthopleura* overlap (Fig. 1). In May, 1985 anemones were also collected from the breakwater at Bodega Bay (northern marine province), and at Arroyo Hondo (southern marine province). The solitary form of *A. elegantissima* (Brandt, 1835) does not occur north of San Francisco, and was therefore not collected at Bodega Bay. *A. xanthogrammica* (Brandt, 1835) is rare south of Pt. Conception (Francis, 1979) and was not present at Arroyo Hondo. Because *A. artemisia* (Dana, 1848) was scarce in the intertidal region at most of the collecting sites, it was collected only at Bodega Bay and Natural Bridges. Natural Bridges has an abundant supply of *A. artemisia* and is close to Davenport Landing, where the other three forms were collected.

At each site, anemones were sampled from the high intertidal down to the low intertidal and along 75 to 150 m of shoreline. Individuals were chosen partly by their availability, but primarily to collect a sample distributed uniformly over the site. For the asexually reproducing forms, single anemones were collected at least 5 m apart to reduce chances of repeatedly sampling the same clone. At each site, 20 anemones of each form present were removed (Table 2) and returned within 1 to 6 h to the laboratory, where they were maintained alive for up to three months in running seawater.

Electrophoresis

The night before an electrophoretic analysis, a small portion of tissue from the tentacles, column, foot or a combination of the three, was cut from each anemone. The samples were homogenized without the addition of buffer and frozen in liquid nitrogen overnight. Thawed samples were analyzed by horizontal starch gel electrophoresis following the standard procedures of Brewer (1970) and Harris and Hopkinson (1978). Two buffers were used: REG (Poulik, 1957) and TMA (buffer number 18; Shaw and Prasad, 1970). The enzymes surveyed with the REG buffer were (standard enzyme abbreviation and number of

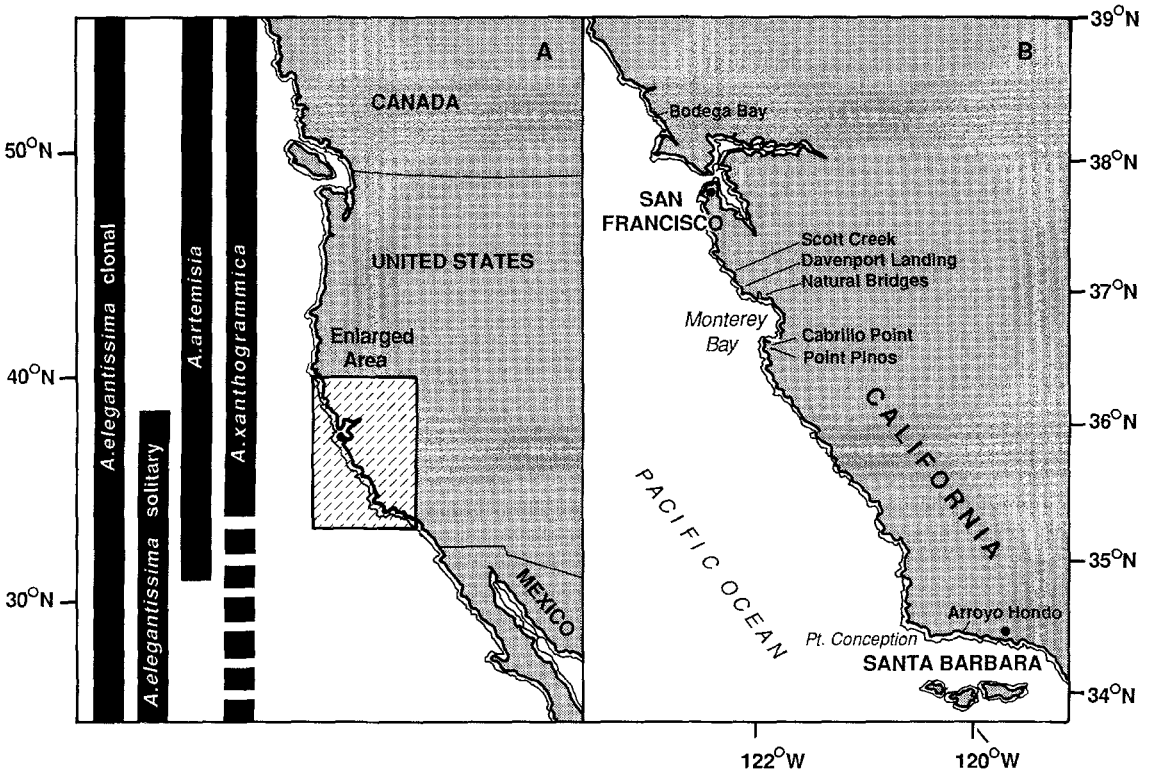

Fig. 1. *Anthopleura* spp. (A) Western North America showing partial ranges of Californian forms after Hand (1955), Francis (1979), Morris *et al.* (1980). (B) Collecting sites in California

Table 2. *Anthopleura* spp. Numbers of individuals collected for each form at seven sites in California, from north to south. Site abbreviations in parentheses. Dash indicates form rare or not found at that site; 0 indicates form present but not collected

Site	Date	No. of individuals			
		A. elegantissima clonal	*A. elegantissima* solitary	*A. xanthogrammica*	*A. artemisia*
Bodega Bay (BB)	May 85	20	–	20	20
Scott Creek (SC)	Mar 85	20	20	20	–
Davenport Landing (DL)	Dec 84	20	20	20	–
Natural Bridges (NB)	May 85	0	0	0	20
Cabrillo Point (CP)	Mar 85	20	20	20	–
Point Pinos (PP)	Dec 84	20	20	20	–
Arroyo Hondo (AH)	May 85	20	20	–	–
Total		120	100	100	40

loci given in parentheses): esterase (EST, 1), leucine amino peptidase (LAP, 1), 6-phosphogluconate dehydrogenase (6-PGDH, 1) and phosphoglucomutase (PGM, 1). The enzymes surveyed with the TMA buffer were: aspartate aminotransferase (AAT, 2), glucose dehydrogenase (GDH, 1), hexokinase (HK, 1), isocitrate dehydrogenase (IDH, 1), malate dehydrogenase (MDH, 2), malic enzyme (ME, 1), mannose phosphate isomerase (MPI, 1), phosphoglucose isomerase (PGI, 2), superoxide dismutase (SOD, 2) and xanthine dehydrogenase (XDH, 1). Detailed recipes for the enzyme stains (Smith, 1986) are based on those of Shaw and Prasad (1970), Ayala *et al.* (1973) and Tracey *et al.* (1975).

All populations listed in Table 2 were analyzed for all of the enzymes, with the exception of the Scott Creek and Cabrillo Point samples, which were not analyzed for PGI. All forms analyzed had the same numbers of loci for each enzyme examined, with one exception: the Mdh-1 locus was not found in *Anthopleura artemisia*. Allozyme patterns were scored following the conventions of Ayala *et al.* (1973). At each locus, one allele was designated "100"; all other alleles were named by adding or subtracting the deviation (in mm) in mobility from this reference allele. The PGI enzyme gave an unusual staining pattern in which the number of bands per individual ranged from 3 to 9. The bands did not appear to be segregating indepen-

dently, and the PGI zymogram was interpreted as two linked loci, as described by Bucklin *et al.* (1984) for other actiniid anemones.

Taxonomic analysis

Nei's genetic identities (I) and distances (D) (Nei, 1972) were computed for all possible comparisons between populations within forms and for all possible comparisons between forms. Sample variances for the genetic distance estimates were computed according to Nei and Roychoudhury (1974), assuming that all anemones sampled were freely inter-breeding, sexual individuals and that the populations were in Hardy-Weinberg equilibrium. Since none of the clonal *Anthopleura elegantissima* or *A. artemisia* had phenotypes identical to those of any other individual sampled within their populations, the first assumption appears valid. To test the significance of genetic distance estimates, χ^2 values were computed for the null hypothesis that the genetic distance between any given pair of populations was zero (Nei and Roychoudhury, 1974).

The sample size was 20 for each form from every collection site where available (Table 2). Since not one intra-form genetic distance was significantly different from zero (see below), the intra-form samples were pooled for the inter-form comparisons.

Genetic variation

Levels of genetic variation within populations were calculated as % polymorphic loci, mean number of alleles and effective number of alleles per locus (equal to $1/1\text{-}H_e$: Ferguson, 1980) and as observed (H_o) and expected (H_e) heterozygosities. To test the agreement of the genotype frequencies with Hardy-Weinberg equilibrium, genotypes were pooled into either homozygote or heterozygote classes and tested using χ^2 (with Yates correction) comparisons of numbers of observed versus expected heterozygotes and homozygotes for each locus. Wright's F-statistics (Wright, 1978) were calculated, using the equations of Hartl (1980), to determine the contributions of within-population heterozygote deficiencies and between-population differentiation to the overall heterozygote deficiency and genetic variation of each species. Wright's first F-statistic (F_{IS} or the population inbreeding coefficient) measures the departure from Hardy-Weinberg proportions observed within local populations. The second F-statistic (F_{ST} or the fixation index) estimates the proportion of heterozygote deficiency in the total sample that is attributable to geographic differentiation among local populations. The final F-statistic (F_{IT} or the overall inbreeding coefficient) measures departure from Hardy-Weinberg proportions in the total population, taking into account the effects of both F_{IS} and F_{ST}.

Results

Species identification

The mean genetic distance between populations of the clonal form of *Anthopleura elegantissima* was .048 while the mean genetic distance between populations of the solitary form was .028 (Table 3). The lowest mean intra-form distance was .009 between populations of *A. xanthogrammica*. The greatest value observed was .107 between the two populations of *A. artemisia*. Neither this value nor any other intra-form genetic distance differed

Table 3. *Anthopleura* spp. Nei's genetic distance (D) ± 1 standard deviation between all possible pairs of populations within each form. Site abbreviations as in Table 2

Populations	Forms			
	A. elegantissima clonal	*A. elegantissima* solitary	*A. xanthogrammica*	*A. artemisia*
BB-SC	.069 ± .01	.045 ± .01	.033 ± .02	
BB-DL	.083 ± .05	.032 ± .04	.005 ± .01	
BB-NB	–	–	–	.107 ± .02
BB-CP	.098 ± .02	.027 ± .01	.005 ± .02	
BB-PP	.046 ± .01	.017 ± .02	.009 ± .01	
BB-AH	.041 ± .04	–	–	
SC-DL	.021 ± .01	.010 ± .01	.012 ± .01	
SC-CP	.069 ± .05	.017 ± .02	.005 ± < .01	
SC-PP	.008 ± .01	.047 ± .03	.014 ± < .01	
SC-AH	.039 ± .02	–	–	
DL-CP	.040 ± .02	.004 ± < .01	< .001 ± < .01	
DL-PP	.040 ± .02	.047 ± .02	.009 ± < .01	
DL-AH	.036 ± .04	–	–	
CP-PP	.050 ± .02	.034 ± .02	.003 ± < .01	
CP-AH	.048 ± .01	–	–	
PP-AH	.030 ± .01	–	–	
Mean	.048 ± .02	.028 ± .02	.009 ± .01	.107 ± .02

Table 4. *Anthopleura* spp. Matrix of Nei's genetic distances (D ± 1 standard deviation) above diagonal and identities (I) below diagonal

Species	*A. elegantissima*			*A. xantho-grammica*	*A. artemisia*
	clonal	solitary	combined		
A. elegantissima					
clonal	–	.016 ± < .01	–	.560 ± .18	.451 ± .16
solitary	.984	–	–	.492 ± .16	.475 ± .16
combined	–	–	–	.518 ± .17	.453 ± .16
A. xanthogrammica	.571	.611	.596	–	.743 ± .19
A. artemisia	.637	.622	.636	.476	–

significantly from zero (χ^2; all $P > 0.05$). Inspection of the table shows no consistent trends of increasing or decreasing genetic distance with latitude or geographic distance between sites, either within or between forms.

Since there was no evidence of population differentiation within forms, the data were pooled for comparisons between forms. The genetic distance between the two forms of *Anthopleura elegantissima* was .016 (Table 4), which is very similar to the mean intra-form genetic distances. These results suggest very strongly that gene flow between the two forms of *A. elegantissima* is not restricted in any way, and that all populations of the two forms are members of the same panmictic species.

Genetic distances between forms other than the two forms of *Anthopleura elegantissima* were more than ten times greater than the mean intra-form genetic distance. The mean genetic distance between *A. xanthogrammica* and *A. elegantissima* was .518, while the mean distance between *A. elegantissima* and *A. artemisia* was .453. The greatest inter-form genetic distance was .743 between *A. xanthogrammica* and *A. artemisia.*

A necessary requirement for identification of species by electrophoresis is that genetic differences between species should not be obscured by genetic variation within the species (Avise, 1975; Ayala, 1983; Thorpe, 1983). To determine whether this could be a problem, the frequency distribution of all possible intra-specific and inter-specific genetic identities (Nei's I) are summarized in Fig. 2. The distribution conforms to an ideal expected bimodal pattern: in every inter-specific comparison, I was less than 0.65; in every intra-specific comparison I was greater than 0.9. Since the contrast between intra-specific and inter-specific comparisons was consistent for all populations, forms and species examined, the identification of species is unambiguous and is not confounded by intra-specific variation.

Although none of the forms was fixed for any allele not found in the other forms, substantial allelic differences were present among comparisons of forms other than the comparison of the two forms of *Anthopleura elegantissima.* The allelic frequencies of the two forms of *A. elegantissima* did not differ significantly at any of the loci surveyed (Smith, 1986). After pooling data from the two forms of *A. elegantissima,* the frequencies of 17 alleles differed by more than .8 (an arbitrarily chosen criterion to judge

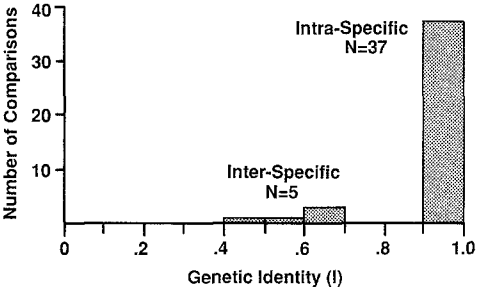

Fig. 2. *Anthopleura* spp. Frequency distribution of Nei's genetic indentities (I) for all possible intra- and inter-specific comparisons

allelic differences), but less than 1.0 among the three species (Table 5). These occurred at four loci between *A. elegantissima* and *A. xanthogrammica,* at two loci between *A. elegantissima* and *A. artemisia* and at six loci between *A. artemisia* and *A. xanthogrammica.*

The uniformly small genetic distances (Table 3) and allelic differences within forms, the lack of differences between clonal and solitary *Anthopleura elegantissima,* the high genetic distances observed among *A. elegantissima, A. xanthogrammica* and *A. artemisia* (Table 4) and the substantial differences in some allelic frequencies (Table 5) all indicate that there are three, and only three, valid species of *Anthopleura* on the coast of California. A cluster analysis of genetic relationships among the forms and species of *Anthopleura* is summarized in Fig. 3. The two forms of *A. elegantissima* are virtually identical, but markedly different from both *A. artemisia* and *A. xanthogrammica,* which, in turn, are very different from each other. *A. xanthogrammica* is the most distant of the three species, while *A. elegantissima* and *A. artemisia* are more similar.

Genetic variation

Five measures of genetic variation within the four forms of *Anthopleura* are presented in Table 6. The proportion of polymorphic loci ranged from 83 to 94%. Loci were considered polymorphic if more than one allele was observed. Of the 18 loci surveyed in the two forms of *A. elegantissima* and *A. xanthogrammica,* only three (Est, Sod-1, Sod-2) were monomorphic in the clonal *A. elegantissima,*

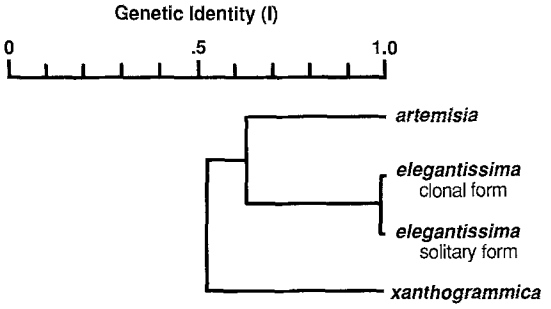

Fig. 3. *Anthopleura* spp. UPGMA dendrogram (Sneath and Sokal, 1973) based on Nei's genetic identities (I), showing genetic relationships among Californian forms

Table 5. *Anthopleura* spp. Gene frequencies for all alleles with frequencies differing by > .8 among species. Dash indicates locus not present

Locus	Allele	*A. elegantissima*	*A. xanthogrammica*	*A. artemisia*
Aat-2	95	.04	.98	.80
	100	.92	.01	.09
Gdh	96	.00	.87	.11
Hk	100	.39	.98	.06
Idh	100	.79	.94	.07
Lap	98	.08	.97	.27
	100	.92	.03	.73
Mdh-1	96	.05	.96	–
	100	.80	.00	–
Mdh-2	95	.00	.94	.03
	100	.86	.06	.96
Me	100	.38	.99	.02
Pgi-1	98	.13	.02	.84
	100	.74	.98	.14
Pgm	100	.15	.99	.04
Sod-2	85	.00	.46	.97
	100	1.00	.54	.03

two (Est, Sod-2) in the solitary *A. elegantissima* and one (Est) in *A. xanthogrammica.* Of the 17 loci surveyed in *A. artemisia,* only one (Est) was monomorphic. Est was the only locus monomorphic in all forms. The mean numbers of alleles per locus among the forms are all within one standard deviation of one another and therefore did not differ significantly. After taking allelic frequencies into account, the effective number of alleles did differ among the forms, with *A. elegantissima* and *A. artemisia* having approximately 35 to 40% more effective alleles than *A. xanthogrammica.*

While observed heterozygosities were approximately half the expected estimates, the relative values of H_o and H_e among the forms were very similar. Both H_o and H_e of *Anthopleura elegantissima* were very similar to those of *A. artemisia.* By these criteria, *A. xanthogrammica* was again the least variable species genetically, with values approximately one third of those for the other two species. Both H_o and H_e of *A. xanthogrammica* were significantly different from the corresponding heterozygosities of *A. elegantissima* and *A. artemisia* ($P < 0.001$; 1-way ANOVAs; arcsine square root transformations of the data). No other comparisons of H_o or H_e among the forms were significant (all $P > 0.05$).

Comparisons of H_o and H_e at each locus within each form are summarized in Table 7, using the combined data from all populations. In one case, a locus was absent in a particular form (Mdh-1 in *Anthopleura artemisia*) and in seven other cases a form was monomorphic ($H_o = .000$) at a particular locus. Among the remaining 64 cases of polymorphic loci, 60 had values of H_o that were lower than expected, and in more than half of these cases (43), the deficiency of heterozygotes was statistically significant (Table 7). When the mean heterozygosities over all loci were considered, there were significant deficiencies of heterozygotes in both *A. elegantissima* and *A. artemisia* ($P < 0.001$). While heterozygote deficiencies were also present at most loci in *A. xanthogrammica,* only six were significant and the mean H_o did not differ significantly from expected. Thus, *A. xanthogrammica* is approximately in Hardy-Weinberg equilibrium, whereas both *A. elegantissima* and *A. artemisia* deviate markedly from equilibrium.

Wright's F-statistics (Wright, 1978) for each form of *Anthopleura* are presented in Table 8. The high F_{IS} values observed in all species of *Anthopleura* reflect the large heterozygote deficiencies observed within most populations. The low F_{ST} values indicate that very little of the heterozygote deficiency can be attributed to differentiation among the local populations sampled within each species. The similarities between the F_{IS} and the F_{IT} values confirm that most of the intra-specific heterozygote deficiency and genetic variation exists within local populations and is not caused by geographic differentiation among the populations sampled.

Discussion

Classification of *Anthopleura*

The data show unequivocally that there are no genetic grounds for treating the two forms of *Anthopleura elegantissima* as anything but members of the same panmictic species. There is no evidence for any restriction of gene flow between the solitary and the clonal forms of *A. elegantissima* and they appear to be fully inter-breeding. It is very unlikely that the similar allelic frequencies found in the two forms could be the result of convergent evolution, random drift or errors in sampling of either genes or individuals. Despite the differing ecology and distributions of the two forms (Francis, 1979), the consistent intra-specific electrophoretic similarities at all loci and among all populations argue against these alternative explanations. Consequently, the hypothesis of Francis (1979) that *A. elegantissima* consists of two sibling species can be rejected.

This classification, using electrophoretic data, is in complete agreement with the classification of Hand (1955) based on general morphology and on nematocyst structure and distributions. There are three, quite distinct Califor-

Table 6. *Anthopleura* spp. Measures of genetic variation (±1 standard deviation)

Species	Total no. loci	Polymorphic loci		No. alleles per locus		Mean heterozygosity	
		Number	%	Mean	Effective	Observed	Expected
A. elegantissima							
clonal	18	15	83.3	3.28±1.45	1.48	.156±.03	.324±.06
solitary	18	16	88.9	3.33±1.33	1.50	.172±.04	.335±.06
combined	18	16	88.9	3.44±1.42	1.49	.154±.03	.328±.06
A. xanthogrammica	18	17	94.4	2.61±.078	1.14	.063±.02	.122±.04
A. artemisia	17	16	94.1	3.29±1.31	1.60	.151±.06	.375±.07

Table 7. *Anthopleura* spp. Observed/expected heterozygosities for 18 loci. Enzyme abbreviations as in Table 3. Asterisks indicate significance of X^2 comparisons of observed with Hardy-Weinberg expectation

Locus	*A. elegantissima* clonal	*A. elegantissima* solitary	*A. xanthogrammica*	*A. artemisia*
Aat-1	.025/.097*	.040/.153**	.000/.019	.100/.431***
Aat-2	.050/.064	.060/.178**	.040/.057	.050/.315***
Est	.000/.000	.000/.000	.000/.000	.000/.000
Gdh	.075/.449***	.070/.328***	.085/.204**	.050/.433***
Hk	.525/.590	.440/.624***	.040/.039	.675/.712
Idh	.075/.348***	.050/.345***	.050/.105	.100/.393***
Lap	.033/.120**	.030/.117*	.010/.047	.050/.397***
Mdh-1	.225/.328*	.200/.321*	.010/.066*	–
Mdh-2	.275/.277	.220/.212	.110/.114	.075/0.73
Me	.250/.570***	.290/.578***	.000/.019	.025/.417***
Mpi	.300/.457***	.510/.727***	.320/.532***	.400/.614**
6-Pgdh	.133/.664***	.130/.573***	.020/.205***	.075/.773***
Pgi-1	.275/.448**	.317/.347	.000/.032	.175/.267
Pgi-2	.300/.385	.317/.419	.016/.016	.250/.560***
Pgm	.133/.570***	.200/.595***	.020/.021	.275/.591***
Sod-1	.000/.000	.010/.028	.010/.076*	.000/.048
Sod-2	.000/.000	.000/.000	.360/.450	.000/.047
Xdh	.142/.411***	.120/.478***	.040/.189	.275/.554**
Mean	.156/.324***	.172/.335***	.063/.122	.151/.375**

– = locus was not found in this species
* = $P<0.05$
** = $P<0.01$
*** = $P<0.001$

Table 8. *Anthopleura* spp. Wright's F-Statistics

Species	F_{IS}	F_{ST}	F_{IT}
A. elegantissima			
clonal	.518	.110	.571
solitary	.487	.077	.526
combined	.530	.116	.585
A. xanthogrammica	.484	.096	.533
A. artemisia	.597	.092	.634

nian species: *Anthopleura elegantissima, A. xanthogrammica* and *A. artemisia.* The three species may be distinguished by a number of gross morphological features, such as disc color and the kind and distribution of verrucae on their columns (Table 1), the distribution and sizes of nematocysts and internal morphology (Hand, 1955), and the frequencies of at least 17 enzymatic alleles (Table 5).

Speciation

Processes of speciation are poorly known among marine organisms (such as *Anthopleura* species) that have high capacities for dispersal. Because of lack of knowledge of the northern and southern boundaries (outside California) of the Californian *Anthopleura* species and of the ranges of other *Anthopleura* species in Japan and Central America, we are restricted in our discussion of possible speciation mechanisms to events on the coast of North America affecting the three Californian species.

In light of their present geographic distribution, *Anthopleura xanthogrammica* and *A. elegantissima* may have diverged as a result of geographic isolation caused by the great changes in the location and size of the California Transition Zone which occurred during the Pliocene and Pleistocene (Newman, 1979; Berggren, 1982; Smith, 1986).

Alternatively, the existence of two ecological forms of *A. elegantissima* suggests another model of sympatric speciation. Today, *A. elegantissima* divides mainly during the fall, which coincides with the time that *A. xanthogrammica* spawns (September to November; Sebens, 1981, 1982). *A. elegantissima* spawns earlier in late August to early October. The optimal time for division by clonal anemones may have forced advancement of their time of spawning, suggesting that reproductive isolation between anemones capable of asexual reproduction (*A. elegantissima*) and strictly sexual anemones (*A. xanthogrammica*) might have evolved as a result of differing times of spawning.

Genetic diversity

The H_e in the strictly sexual *Anthopleura xanthogrammica* (.122) is similar to the average levels of genetic diversity observed in many other sexually reproducing coelenterates and other marine invertebrates (Bucklin and Hedgecock, 1982; Nevo *et al.*, 1984). By contrast, values of H_e in the asexual and sexual *A. artemisia* (.375) and *A. elegantissima* (.328) are very high. Even higher values of H_e have been reported in some other anemone species, e.g. .410 in *Urticina felina* and .436 in *U. eques* (Sole-Cava *et al.*, 1985), but it is not known whether these anemones are clonal (Sole-Cava *et al.*, 1985).

Although the procedure used to collect anemones in this study minimized clonal duplication and was not designed to examine intra-population structures, the extent of clonal variation of *Anthopleura elegantissima* differed noticeably from that reported in other anemones, such as *Actinia tenebrosa* (Ayre, 1984), *Haliplanella luciae* (Shick and Lamb, 1977) and, to a varying degree, *Metridium senile* (Hoffmann, 1986). Populations of the latter species typically consist of large numbers of individuals of a few genotypes that dominate large areas (entire sites). In contrast, no instance of a repeated genotype was observed in any of the *A. elegantissima* populations we sampled over areas averaging less then 4 000 square meters. Each *A. elegantissima* population consisted of many clones containing relatively few individuals. A number of factors may explain this dramatic difference in clonal structure between the species of anemones, including differences in modes of asexual reproduction (*A. tenebrosa* and *M. senile*) and ecology (*H. luciae*).

The heterozygosity data (Table 7) show large heterozygote deficiencies from Hardy-Weinberg expectations at almost every locus sampled in *Anthopleura elegantissima* and *A. artemisia*. Heterozygote deficiencies are characteristic of many sessile marine invertebrates (Berger, 1983; Koehn and Gaffney, 1984; Zouros and Foltz, 1984; Mallet *et al.*, 1985). The degree of heterozygote deficiency observed in *A. elegantissima* and *A. artemisia* is similar to that observed in the oyster *Crassostrea virginica* (Zouros *et al.*, 1980) and the gastropod *Thais haemastoma* (Garton, 1984). The possible causes of this heterozygote deficiency include both population characteristics of the anemones as well as possible experimental biases of electrophoresis. These varied factors have been reviewed in many other electrophoretic studies (Zouros *et al.*, 1980; Berger, 1983; Zouros and Foltz, 1984; Mallet *et al.*, 1985). The possibility of null alleles has been raised recently (Foltz, 1986), although the consistent scoring patterns and heterozygote deficiencies do not support this hypothesis in the case of *Anthopleura* species. The presence of heterozygote deficiencies at virtually all loci in *Anthopleura* species argues for a population structural mechanism rather than selection or differential mortality as the most probable cause of the deficiency.

Given the lack of population differentiation observed in *Anthopleura* species and the scale of sampling used in collecting the anemones, a geographic Wahlund effect (the mixing of larvae from differentiated but locally panmictic populations leading to overestimates of genetic diversity) is an unlikely cause of the heterozygote deficiency. An alternative mechanism for producing the Wahlund effect may be the localized fertilization of gametes before larval dispersal. Higher probabilities of mating with near neighbors would cause the effective panmictic gamete pool to be a small, non-random sample of the entire population. Any gene present locally at frequencies above the population frequency will produce more homozygotes than expected from the population Hardy-Weinberg equilibrium, even if there are no barriers to dispersal of gametes and larvae. A spatial Wahlund effect due to local probabilities of random fertilization may be a cause of the heterozygote deficiencies observed in *Anthopleura* species.

The potential for extended lifespans in clonal organisms has been well documented in corals (Nozaki *et al.*, 1978; Druffel, 1982; Potts *et al.*, 1985) and terrestrial plants (Vasek, 1980; Cook, 1983). Once a large clone of anemones is established, the clonal genotype may persist for a very long time. This extended genotype lifespan may account for the high genetic diversity found in *Anthopleura artemisia* and *A. elegantissima*. It has been suggested that prolonged, overlapping generations in long-lived organisms with mixed modes of reproduction may create a buffer against the decay of genetic variation (Hiebert, 1977; Hamrick, 1979; Hamrick *et al.*, 1979): an effect that may be intensified by the persistence and growth of successful clones (Levin, 1978; Potts, 1984). A correlation between longevity and increased gene diversity over a wide range of organisms has been reported by Hamrick *et al.* (1979) and Nevo *et al.* (1984). Like long-lived perennial plants, *A. artemisia* and *A. elegantissima* may maintain large stores of genetic diversity through the extended longevity and repeated reproduction of large, old clones.

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