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C. S. McFadden · R. K. Grosberg · B. B. Cameron
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Genetic relationships within and between clonal and solitary forms of the sea anemone *Anthopleura elegantissima* revisited: evidence for the existence of two species

Abstract Along the temperate Pacific coast of North America, the actiniarian sea anemone *Anthopleura elegantissima* exhibits two discrete life-history phenotypes. Although both forms sexually produce planula larvae, the clonal morph can also asexually propagate by fission, whereas the solitary morph does not. Whether the two forms constitute one or two species has long been contested. Hand originally designated the two forms as conspecifics, whereas Francis – on the basis of differences in microhabitat, biogeographic range and phenotypic frequencies – argued that the two forms constituted a sibling-species pair. From the results of an electrophoretic survey in which they pooled allelic frequencies across several geographic locations, Smith and Potts subsequently argued that the two forms were not genetically differentiated, and therefore represented a single species. We re-examined the relationship between the forms electrophoretically, substantially extending the geographic range and doubling the sample sizes beyond those used by Smith and Potts, and not pooling allelic frequencies in our analyses. Our analysis of patterns of genetic variation at ten highly polymorphic allozyme loci shows that although no fixed genetic differences distinguish the two forms, there are significant differences in allele frequencies between clonal and solitary *A. elegantissima* at every site we sampled throughout their range of sympatry (over 1000 km); within each form, however, there is little detectable genetic differentiation among populations. We therefore conclude that the two forms represent recently reproductively isolated taxa, and propose that the clonal form retain the binomial *A. elegantissima* (Brandt, 1835), whereas the solitary form be described and named a new species, *Anthopleura* sp.

C.S. McFadden (✉) · D.P. Karlton[1]
Harvey Mudd College, Department of Biology,
Claremont, California 91711, USA

R.K. Grosberg · B.B. Cameron
Center for Population Biology, University of California,
Davis, California 95616, USA

D. Secord
University of Washington, Tacoma Liberal Studies,
Perkins Building, 1103 A Street, Tacoma,
Washington 98402, USA

Present address:
[1]7503 De Foe Drive, Cupertino,
California 95014, USA

Introduction

The life cycles of many sea anemones regularly feature, along with sexual reproduction, some form of asexual propagation (reviews by Chia 1976; Shick 1991). The occurrence and mode of asexual propagation – whether via budding, fission, pedal laceration, or apomictic parthenogenesis – varies among families, genera, and even sister-species within the same genus (Chia 1976; Francis 1988; Shick 1991), suggesting that asexual multiplication has a complex evolutionary history among anemones. For example, some species in the genus *Metridium* clone throughout their life cycles, whereas other congeners never clone, living as solitary individuals, each the unique genetic product of a single sexually produced zygote (Bucklin 1987). Similar patterns of life-history variation occur in other genera of anemones, such as *Actinia* and *Epiactis* (reviews by Hughes 1989; Shick 1991; see also Edmands 1995). Such fundamental differences in life history are often the primary characters used to distinguish closely related species of anemones (Bucklin and Hedgecock 1982; Bucklin 1987), and species in other invertebrate taxa as well (e.g. Mladenov and Emson 1990). However, like many facultatively asexual organisms (reviewed by Hughes 1989), members of a given species of anemone can exhibit very different life histories, as different as clonal versus solitary, in response to a combination of genetic and environmental variation (e.g. Sebens 1979, 1980; Shick et al. 1979; Bucklin 1985; Lin et al. 1992;

Tsuchida and Potts 1994a, b). Consequently, genetic markers, whose expression is not subject to environmental modification, must be used to assess the extent to which forms exhibiting different life histories are reproductively isolated and therefore represent distinct taxa (Bucklin and Hedgecock 1982; Bucklin 1985).

Along the west coast of North America, the anemone *Anthopleura elegantissima* (Brandt) inhabits wave-swept rocky shores from Alaska (Hand 1955) south at least to central Baja California (Grosberg and Secord personal observations). Hand first described two morphologically and ecologically distinct forms of *A. elegantissima* that live sympatrically from central California southward (Hand 1955; Francis 1979). Both forms are gonochoric and free-spawning, producing long-lived (at least several weeks) planktonic larvae (Ford 1964; Siebert 1974; Jennison 1979). The clonal form, however, also propagates asexually by longitudinal fission, forming tightly packed aggregations of relatively small polyps (reviewed by Francis 1979). The polyps of the solitary form, on the other hand, are much larger than those of the clonal form, and do not divide (Francis 1979). In addition, although the two forms may live syntopically, the clonal form occurs more often on the tops of boulders exposed to strong surf, whereas the solitary form occupies more protected aspects of such boulders and generally occurs lower on the shore, sometimes lying partially buried in sand (Francis 1979; Smith and Potts 1987; J.S. Pearse personal communication; McFadden personal observations).

Based on these differences in polyp size, biogeographic range and habitat, as well as differences in frequencies of color patterns on the column, oral disk, and tentacles, Francis (1979) proposed that the two forms of *Anthopleura elegantissima* are actually reproductively isolated species. However, habitat differences have been shown to cause dramatic variation in life-history (and many other) traits in this and other species of anemone (Sebens 1979, 1980; Ayre 1984; Bucklin 1985; Shick 1991; Lin et al. 1992; Tsuchida and Potts 1994a, b), and Smith and Potts (1987) later argued on the basis of allozyme data from 15 polymorphic loci that the two forms of *A. elegantissima* could not be distinguished genetically and hence could not be called different species. Smith and Potts drew this conclusion based on relatively small differences in genetic distance between the forms compared to the distances between co-occurring congeners, including *A. xanthogrammica* and *A. artemisia*. These data remain difficult to evaluate for several reasons. First, the sample sizes examined by Smith and Potts were small, comprising only 20 individuals of each form from six locations in the northern half of their range of sympatry. Second, in the process of making the between-form genetic comparison, they pooled samples of each form from different locations, potentially obscuring any geographic variation underlying differences in allelic frequencies between forms.

Given that the clonal and solitary *Anthopleura elegantissima* express very different life histories while living side-by-side, we re-examined the long-standing question (e.g. Francis 1979; Smith and Potts 1987) of genetic relationships within and between the two forms. With respect to the study of Smith and Potts, we substantially extended the geographic range of sampling, as well as the number of individuals of both forms sampled at each site. In addition, we based our analysis on statistical comparisons of allelic frequencies and genetic distances between forms within each site, rather than on relative genetic distance measures among forms pooled across different geographic locations.

Materials and methods

Sampling sites and protocols

During the summers of 1993 and 1994, and in March 1995, we collected tissue samples from clonal and solitary *Anthopleura elegantissima* polyps at each of 11 sites along the NE Pacific coast, from northern California (USA) to Baja California (Mexico) (Fig. 1). In addition, we collected clonal *A. elegantissima* from two sites in Oregon (Fig. 1), located north of the geographic range of the solitary form. Four collection sites (Doran Rocks, Hopkins Marine Station, Point Sal, Gaviota) lie within the same geographic range and close to sites included in Smith and Potts' (1987) study. At each site we collected tissue from 40 individuals of each form that was present, with the exception of Punta Banda (PB), where the clonal form was uncommon and we were able to obtain only 17 samples.

We distinguished clonal from solitary individuals on the basis of polyp size and proximity to other individuals. We considered individuals to be clonal which were members of dense stands of polyps with similar color markings (Francis 1973a, 1979), and whose pedal disks had diameters less than $\simeq 2.5$ cm. We considered polyps to be solitary if they had pedal disk diameters >3 cm and lacked neighbors within tentacular reach. To reduce the possibility of repeatedly sampling the same clone, we gathered single clonal individuals from aggregations on boulders isolated from other rocks by uninhabitable substrate; along shorelines that lacked isolated boulders, we collected from aggregations separated by a distance of at least 5 m. Whenever possible, we sampled tissue from the polyp of the solitary individual nearest to each of the collected clonal individuals, thereby minimizing the effects that microhabitat differences might have on genetic differentiation among forms at a site.

For clonal individuals, we usually pried the entire polyp from the substrate. For solitary individuals, we cut a small piece of tissue (~ 100 to 200 mm^3) from the foot, or when foot tissue was not accessible, from the oral disk, tentacles, or body wall. We cleaned the samples of attached debris and opened the coelenterons of clonal individuals to reduce potential contamination by undigested food. Samples were then placed in individual vials and frozen in liquid nitrogen at the collecting site or returned to the laboratory on ice and frozen in a -80 °C freezer.

Electrophoresis

We prepared samples for horizontal starch-gel electrophoresis by homogenizing a small piece of tissue (~ 70 to 160 mg) with an approximately equal volume of finely ground glass (from broken coverslips) and 60 μl grinding buffer (0.05 M Tris-HCl, pH 7.0) (Black and Johnson 1979). We then soaked paper wicks in the homogenate and inserted them into 12% starch (Sigma) gels.

Following the procedures in Ayre and Grosberg (1995), we initially screened anemones for 11 enzyme systems and a total of 16 variable enzyme-encoding loci: malate dehydrogenase (*MDH-1*, *MDH-2*; E.C. 1.1.1.37), octopine dehydrogenase (*ODH*; E.C.

from two loci overlapped so extensively on the gels that we could not dependably score genotypes (*LAP-1* and *LAP-2*).
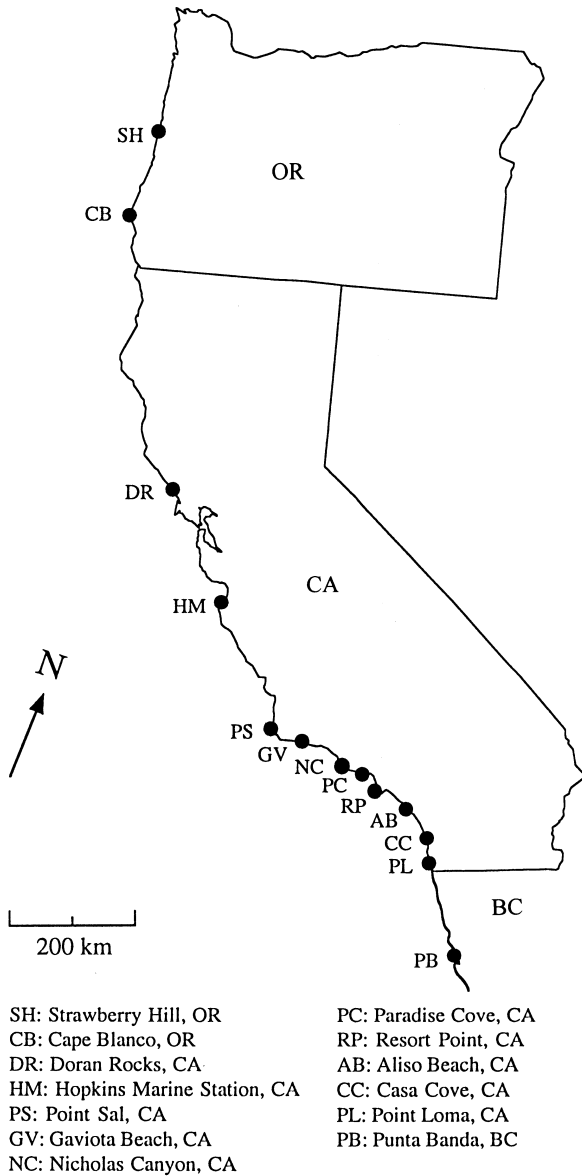
Analysis

We compiled allelic and genotypic frequencies separately for each form of *Anthopleura elegantissima* at each site and, after pooling rare alleles (Lessios 1992), used a chi-square test for heterogeneity (BIOSYS-1; Swofford and Selander 1981) to compare allelic frequencies between clonal and solitary populations at each site. We also estimated the mean number of alleles per locus for each form at each site, as well as the mean observed heterozygosity per locus (direct count) and mean Hardy–Weinberg expected heterozygosity per locus [Nei's (1978) unbiased estimate]. We report deviations between observed (H_o) and expected (H_e) heterozygosity estimates at each locus in each form and at each site as $D = (H_o - H_e)/H_e$.

To determine if genotypic frequencies at each locus in each form conformed to Hardy–Weinberg expectations, we used chi-square goodness-of-fit tests. For loci with low expected frequencies in one or more genotypic classes, we pooled rare alleles or (for diallelic loci) used exact probabilities (BIOSYS-1; Swofford and Selander 1981). For these tests and for the chi-square tests for heterogeneity of allele frequencies, we adjusted significance levels for multiple tests using Hochberg's (1988) modification of the Bonferroni procedure (Lessios 1992).

To characterize patterns of genetic structure among sampled populations of each form, we calculated Weir and Cockerham's (1984) estimators of Wright's (1978) *F*-statistics, using the program "Genetic Data Analysis" (Lewis and Zaykin 1996). The estimators F, f, and θ, correspond respectively to F_{IT} (total inbreeding), F_{IS} (component due to inbreeding within each sample), and F_{ST} (component due to subdivision among sampled populations). We estimated the 95% confidence intervals about the mean estimators by bootstrapping across loci, and calculated the standard errors of the mean by jackknifing over loci (Weir 1990, 1996).

Finally, because the two forms of *Anthopleura elegantissima* could represent evolutionarily distinct taxa, we estimated the genetic distances among populations using Cavalli-Sforza's chord distance (Cavalli-Sforza and Edwards 1967; Felsenstein 1993: PHYLIP Version 3.52c). Unlike more commonly used metrics [e.g. Nei's (1972) or Rogers' (1972) genetic distances], the Cavalli-Sforza metric is relatively insensitive to variation in evolutionary rates among loci and lineages (Swofford and Olsen 1990; Cunningham and Collins 1994). We used the distances to construct a strict consensus, majority-rule neighbor-joining tree, based on 200 bootstrapped iterations (Felsenstein 1993: PHYLIP Version 3.52c).

Results

Genetic relationships of clonal and solitary forms of *Anthopleura elegantissima*

The number of unique multilocus genotypes detected among the 40 clonal individuals collected at each site (excluding Punta Banda, PB) ranged from 39 (at Strawberry Hill, SH) to only 18 (at Hopkins Marine Station, HM), with the majority of samples comprising >30 unique genotypes (Table 1). At PB we found only 9 unique genotypes among 17 clonal individuals collected. From the observed allele frequencies we estimated the expected frequency of the most common multilocus genotype for the clonal form at each site, assuming sexual recombination and linkage equilibrium. The probability of two clonal individuals in the same population sharing the most common multilocus genotype as a result of

Fig. 1 *Anthopleura elegantissima*. Locations of populations sampled along NE Pacific coast from Oregon (*OR*) to Baja California (*BC*) (*CA* California)

SH: Strawberry Hill, OR
CB: Cape Blanco, OR
DR: Doran Rocks, CA
HM: Hopkins Marine Station, CA
PS: Point Sal, CA
GV: Gaviota Beach, CA
NC: Nicholas Canyon, CA
PC: Paradise Cove, CA
RP: Resort Point, CA
AB: Aliso Beach, CA
CC: Casa Cove, CA
PL: Point Loma, CA
PB: Punta Banda, BC

1.5.1.11), 6-phosphogluconate dehydrogenase (*6 PGD*; E.C. 1.1.1.44), xanthine oxidase (*XO*; E.C. 1.2.3.2), leucine amino peptidase (*LAP-1*, *LAP-2*; E.C. 3.4.11), hexokinase (*HK*; E.C. 2.7.1.1), leucyl alanine peptidase (*PEP-1*, *PEP-2*; E.C. 3.4.11), phosphoglucose isomerase (*PGI-1*, *PGI-2*; E.C. 5.3.1.9), phosphoglucomutase (*PGM*; E.C. 2.7.5.1), isocitrate dehydrogenase (*IDH-1*, *IDH-2*; E.C. 1.1.1.42), and mannose phosphate isomerase (*MPI*; E.C. 5.3.1.8). We ran *MDH*, *ODH* and *6 PGD* on Buffer System 5 (tris citrate pH 8.0), *XO*, *LAP*, *HK*, and *PEP* on Buffer System 6 (tris-EDTA-borate pH 8.0), and the remaining loci on Buffer System 9 (tris-maleate pH 7.4) of Selander et al. (1971).

We numbered loci and lettered alleles in order of decreasing electrophoretic mobility. After a preliminary screening of the enzyme systems listed above, we found we could reliably score eight systems encoding ten loci. We excluded the remaining six loci from further analysis because (1) band intensity and resolution varied extensively within and among samples (*XO*, *PEP-1*, *PGI-1*); (2) null alleles appeared to be so common that we could not infer genotypic frequencies reliably (*ODH*, *LAP-1*, and *LAP-2*); or (3) the products

Table 1 *Anthopleura elegantissima*. Allele frequencies in populations of clonal (*CL*) and solitary (*SO*) forms collected from 13 sites along NE Pacific coast [*N* number of individuals or clones analyzed for each locus; *site abbreviations* (= populations) as in Fig. 1; χ^2 *values* refer to tests of heterogeneity of allele frequencies between forms at a site; *df* degrees of freedom; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$]

Locus, allele	SH	CB	DR		HM		PS		GV		NC		PC		RP		AB		CC		PL		PB	
	CL	CL	CL	SO	CL	SO	CL	SO	CL	SO	CL	SO	CL	SO	CL	SO	CL	SO	CL	SO	CL	SO	CL	SO
MDH-1																								
(*N*)	39	35	32	39	18	39	33	39	35	39	32	40	36	40	25	40	29	40	38	40	32	40	9	40
A	0.013	0.000	0.141	0.064	0.361	0.115	0.061	0.128	0.057	0.103	0.094	0.125	0.014	0.162	0.040	0.125	0.000	0.162	0.000	0.138	0.031	0.100	0.056	0.050
B	0.987	1.000	0.859	0.821	0.639	0.756	0.924	0.705	0.943	0.808	0.906	0.738	0.986	0.700	0.960	0.762	1.000	0.663	0.987	0.725	0.953	0.850	0.833	0.825
C	0.000	0.000	0.000	0.103	0.000	0.090	0.015	0.154	0.000	0.077	0.000	0.138	0.000	0.100	0.000	0.100	0.000	0.162	0.013	0.100	0.016	0.038	0.111	0.075
D	0.000	0.000	0.000	0.013	0.000	0.038	0.000	0.013	0.000	0.013	0.000	0.000	0.000	0.038	0.000	0.013	0.000	0.013	0.000	0.038	0.000	0.013	0.000	0.050
χ^2(*df*)			0.39 (1)		1.69 (1)		10.96 (1)		6.02 (1)		10.36 (2)		22.70 (2)***		8.86 (1)		24.34 (2)***		21.42 (2)**		4.05 (1)		0.01 (1)	
MDH-2																								
(*N*)	39	35	32	38	18	40	33	40	34	39	31	40	35	40	24	40	28	40	38	40	32	40	9	40
A	0.808	0.786	0.813	0.829	0.972	0.800	0.879	0.850	0.941	0.808	0.935	0.813	0.957	0.825	1.000	0.762	0.964	0.825	1.000	0.825	1.000	0.850	1.000	0.788
B	0.192	0.214	0.188	0.171	0.028	0.200	0.121	0.150	0.059	0.192	0.065	0.188	0.043	0.175	0.000	0.237	0.036	0.175	0.000	0.175	0.000	0.150	0.000	0.213
χ^2(*df*)			0.06 (1)		5.89 (1)		0.25 (1)		5.72 (1)		4.56 (1)		6.49 (1)		13.39 (1)*		6.16 (1)		14.61 (1)*		10.47 (1)		4.63 (1)	
6PGD																								
(*N*)	35	35	32	38	18	39	34	40	35	39	32	40	36	40	25	40	29	40	38	40	32	40	9	40
A	0.090	0.129	0.141	0.000	0.167	0.000	0.015	0.013	0.000	0.013	0.000	0.013	0.014	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.013
B	0.846	0.714	0.750	0.842	0.778	0.859	0.971	0.863	0.986	0.872	1.000	0.850	0.986	0.863	1.000	0.825	1.000	0.813	1.000	0.837	1.000	0.850	0.944	0.850
C	0.064	0.157	0.109	0.079	0.056	0.115	0.015	0.112	0.014	0.077	0.000	0.087	0.000	0.087	0.000	0.075	0.000	0.112	0.000	0.087	0.000	0.100	0.000	0.087
D	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.013	0.000	0.000	0.000	0.013	0.000	0.000
E	0.000	0.000	0.000	0.066	0.000	0.026	0.000	0.013	0.000	0.038	0.000	0.050	0.000	0.038	0.000	0.087	0.000	0.063	0.000	0.075	0.000	0.038	0.000	0.050
χ^2(*df*)			1.84 (1)		1.17 (1)		5.36 (1)		6.96 (1)		10.47 (1)		7.96 (1)		9.81 (1)		12.20 (1)*		13.47 (1)*		10.47 (1)		1.14 (1)	
HK																								
(*N*)	39	35	32	39	18	38	34	40	34	38	32	40	36	40	25	40	29	40	36	39	32	39	9	38
A	0.000	0.000	0.000	0.013	0.000	0.013	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.013	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.014	0.000	0.115	0.000	0.158	0.000	0.188	0.044	0.171	0.000	0.162	0.014	0.138	0.000	0.350	0.000	0.175	0.000	0.141	0.016	0.244	0.000	0.158
C	0.154	0.257	0.203	0.487	0.167	0.382	0.059	0.325	0.088	0.434	0.219	0.475	0.153	0.512	0.120	0.375	0.172	0.363	0.292	0.487	0.313	0.436	0.056	0.461
D	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.846	0.700	0.781	0.321	0.750	0.303	0.941	0.300	0.868	0.289	0.781	0.275	0.833	0.287	0.880	0.213	0.828	0.363	0.694	0.295	0.656	0.205	0.944	0.276
F	0.000	0.000	0.016	0.064	0.083	0.118	0.000	0.125	0.000	0.092	0.000	0.087	0.000	0.038	0.000	0.025	0.000	0.063	0.014	0.077	0.016	0.103	0.000	0.066
G	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.025	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
H	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.026
χ^2(*df*)			31.77 (2)***		20.04 (2)***		63.76 (3)***		48.80 (2)***		40.69 (2)***		46.75 (2)***		46.91 (1)***		33.28 (2)***		21.74 (2)***		36.86 (3)***		27.04 (2)***	
PEP-2																								
(*N*)	39	35	32	39	18	40	34	39	32	39	27	40	36	35	25	40	29	40	38	40	32	40	9	40
A	0.590	0.414	0.422	0.128	0.333	0.338	0.368	0.282	0.203	0.231	0.315	0.162	0.208	0.271	0.220	0.150	0.155	0.287	0.263	0.200	0.344	0.112	0.500	0.412
B	0.256	0.386	0.156	0.385	0.194	0.100	0.309	0.192	0.469	0.308	0.333	0.412	0.292	0.243	0.400	0.338	0.345	0.313	0.329	0.262	0.313	0.275	0.500	0.213
C	0.154	0.171	0.422	0.436	0.472	0.563	0.324	0.526	0.328	0.462	0.352	0.425	0.500	0.486	0.380	0.512	0.500	0.400	0.408	0.538	0.344	0.613	0.000	0.375
D	0.000	0.029	0.000	0.051	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
χ^2(*df*)			13.68 (2)		0.81 (1)		6.27 (2)		4.08 (2)		4.31 (2)		0.92 (2)		2.36 (2)		3.41 (2)		2.64 (2)		14.21 (2)		11.59 (2)	

The following allozyme data table is printed sideways on the page. It is transcribed here with allele loci/rows and population samples as columns (1–24).

PGI-1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
(N)	39	35	32	38	7	28	34	35	32	34	36	32	40	36	39	23	39	29	38	35	32	36	9	22
A	0.128	0.186	0.203	0.171	0.000	0.125	0.088	0.114	0.000	0.147	0.262	0.078	0.262	0.042	0.192	0.000	0.231	0.000	0.237	0.224	0.016	0.111	0.278	0.205
B	0.744	0.743	0.656	0.829	0.786	0.857	0.632	0.829	0.469	0.794	0.725	0.422	0.725	0.500	0.795	0.674	0.718	0.603	0.711	0.763	0.516	0.819	0.444	0.750
C	0.128	0.071	0.141	0.000	0.214	0.018	0.279	0.057	0.531	0.059	0.013	0.500	0.013	0.458	0.013	0.326	0.051	0.397	0.053	0.013	0.469	0.069	0.278	0.045

$\chi^2(df)$ values reported (column order): 5.53 (1); 12.27 (2); 40.46 (2)***; 49.10 (2)***; 44.85 (2)***; 24.96 (2)***; 33.62 (2)***; 39.42 (2)***; 14.29 (1)*; 5.34 (1)

PGM

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
(N)	39	35	32	39	18	40	34	40	35	39	34	32	40	34	37	25	38	40	40	38	32	40	39	39
A	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.038	0.157	0.094	0.103	0.028	0.050	0.265	0.112	0.071	0.077	0.118	0.031	0.125	0.118	0.122	0.160	0.079	0.241	0.150	0.132	0.094	0.188	0.056	0.205
C	0.564	0.357	0.297	0.462	0.500	0.550	0.338	0.400	0.486	0.410	0.397	0.609	0.500	0.397	0.486	0.520	0.474	0.431	0.387	0.487	0.484	0.450	0.667	0.487
D	0.231	0.386	0.484	0.397	0.472	0.387	0.382	0.475	0.371	0.474	0.368	0.328	0.325	0.368	0.311	0.300	0.382	0.310	0.425	0.316	0.313	0.363	0.111	0.231
E	0.167	0.100	0.125	0.026	0.000	0.013	0.015	0.013	0.071	0.038	0.103	0.031	0.050	0.103	0.081	0.020	0.053	0.017	0.038	0.063	0.109	0.000	0.167	0.026
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.051

$\chi^2(df)$ values reported (column order): 4.28 (2); 5.58 (2); 1.61 (2); 1.46 (3); 4.38 (2); 0.94 (2); 2.11 (2); 1.14 (2); 0.40 (2); 1.89 (1)

IDH-1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
(N)	38	35	32	39	18	40	33	40	35	39	36	32	40	36	40	25	40	40	40	38	32	40	9	40
A	0.000	0.000	0.000	0.128	0.000	0.088	0.029	0.162	0.014	0.179	0.000	0.000	0.075	0.000	0.038	0.000	0.063	0.000	0.188	0.000	0.016	0.100	0.000	0.087
B	1.000	1.000	1.000	0.872	1.000	0.912	0.971	0.837	0.986	0.808	1.000	1.000	0.913	1.000	0.962	1.000	0.938	1.000	0.788	1.000	0.984	0.900	1.000	0.900
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.013

$\chi^2(df)$ values reported (column order): 8.83 (1); 3.37 (1); 7.15 (1); 12.13 (1)*; 5.89 (1); 2.75 (1); 3.25 (1); 6.16 (1); 18.13 (1)**; 4.32 (1); 1.96 (1); 0.25 (1)

IDH-2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
(N)	38	35	32	39	18	35	33	39	35	35	36	32	40	36	40	25	40	40	37	37	32	37	9	40
A	0.053	0.000	0.016	0.026	0.250	0.043	0.147	0.064	0.157	0.071	0.125	0.188	0.038	0.125	0.038	0.180	0.013	0.086	0.108	0.405	0.047	0.000	0.000	0.075
B	0.947	0.986	0.984	0.897	0.750	0.929	0.853	0.833	0.843	0.900	0.875	0.813	0.887	0.875	0.875	0.820	0.950	0.914	0.892	0.581	0.953	0.959	1.000	0.900
C	0.000	0.014	0.000	0.077	0.000	0.029	0.000	0.103	0.000	0.029	0.000	0.000	0.075	0.000	0.087	0.000	0.038	0.000	0.000	0.014	0.000	0.041	0.000	0.025

$\chi^2(df)$ values reported (column order): 4.29 (1); 6.61 (1); 0.11 (1); 1.02 (1); 1.61 (1); 0.00 (1); 5.78 (1); 0.72 (1); 18.42 (1)**; 0.03 (1); 1.96 (1)

MPI

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
(N)	39	35	32	39	18	39	33	40	35	39	36	32	40	36	40	25	40	29	38	37	32	40	9	40
A	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.013	0.000	0.013	0.000	0.000	0.013	0.000	0.000	0.000	0.013	0.013	0.000	0.013	0.000	0.013	0.000	0.013
B	0.000	0.000	0.000	0.013	0.013	0.025	0.000	0.025	0.000	0.013	0.000	0.000	0.025	0.000	0.000	0.000	0.013	0.000	0.000	0.013	0.000	0.013	0.056	0.013
C	0.000	0.000	0.000	0.013	0.038	0.013	0.000	0.013	0.051	0.013	0.000	0.025	0.025	0.000	0.050	0.000	0.013	0.013	0.000	0.063	0.000	0.038	0.000	0.013
D	0.397	0.286	0.313	0.103	0.231	0.213	0.167	0.213	0.071	0.077	0.028	0.138	0.138	0.028	0.100	0.120	0.162	0.000	0.013	0.112	0.000	0.162	0.000	0.213
E	0.487	0.586	0.594	0.308	0.244	0.313	0.742	0.313	0.871	0.295	0.889	0.313	0.938	0.889	0.237	0.860	0.237	0.966	0.974	0.438	0.953	0.350	1.000	0.250
F	0.051	0.043	0.063	0.256	0.179	0.188	0.000	0.188	0.000	0.231	0.000	0.188	0.016	0.000	0.250	0.000	0.275	0.000	0.000	0.188	0.000	0.188	0.000	0.175
G	0.013	0.014	0.000	0.115	0.000	0.125	0.014	0.175	0.000	0.192	0.028	0.000	0.000	0.028	0.175	0.000	0.100	0.000	0.000	0.063	0.031	0.100	0.000	0.162
H	0.051	0.071	0.031	0.128	0.028	0.128	0.043	0.100	0.043	0.115	0.056	0.047	0.047	0.056	0.112	0.020	0.125	0.034	0.013	0.075	0.016	0.138	0.000	0.112
I	0.000	0.000	0.000	0.051	0.013	0.013	0.000	0.013	0.000	0.026	0.000	0.000	0.000	0.000	0.013	0.000	0.025	0.000	0.000	0.025	0.000	0.000	0.000	0.025
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000
K	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L	0.000	0.000	0.000	0.000	0.013	0.013	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

$\chi^2(df)$ values reported (column order): 38.86 (4)***; 48.74 (3)***; 38.37 (4)***; 53.72 (4)***; 58.61 (4)***; 68.69 (5)***; 52.91 (3)***; 70.48 (4)***; 53.47 (3)***; 55.54 (4)***; 34.82 (2)**

132

sexual recombination ranged from only 9×10^{-3} to 5×10^{-5} (depending on the expected heterozygosity of the population); consequently, we assumed that any individuals sampled from the same site that shared the same multilocus genotype were most likely clonemates, and eliminated all but one individual of each clone from subsequent calculations of allelic and genotypic frequencies (Table 1). With only two exceptions, all the solitary individuals collected from each site had unique multilocus genotypes.

Genotype frequencies within populations of both forms deviated significantly from Hardy–Weinberg expectations in only 13 of 217 comparisons, and the number of deficits of heterozygotes approximately equaled the number of heterozygote excesses (Table 2). In 23 of 24 observations, however, the $PEP-2$ locus exhibited large heterozygote deficiencies, and 12 of the 13 significant deviations from Hardy–Weinberg expected frequencies were for this locus.

At all 11 sites from which we collected both clonal and solitary forms of *Anthopleura elegantissima,* we found significant differences in allele frequencies between forms at two or more loci (heterogeneity chi-square, $p < 0.05$; Table 1). Specifically, allelic frequencies differed significantly between clonal and solitary forms for $PGI-1$ at 7 of 11 sites, and for HK and MPI at all 11 sites (Table 1).

Table 2 *Anthopleura elegantissima.* Heterozygote deficiencies (D) within populations of clonal (*CL*) and solitary (*SO*) forms. Significance values are shown for loci at which genotype frequencies deviated from Hardy–Weinberg expectations [significance levels adjusted using Hochberg's (1988) Bonferroni technique (see ''Materials and methods – Analysis''): $^{*}p < 0.05$; $^{**}p < 0.01$] (– locus not polymorphic in a population; *site abbreviations* as in Fig. 1)

Site, form	Locus									
	MDH-1	MDH-2	6PGD	HK	PEP-2	PGI-1	PGM	IDH-1	IDH-2	MPI
SH										
CL	0.013	−0.092	0.037	0.182	−0.317	0.114	−0.059	–	0.056	−0.187
CB										
CL	–	−0.236	0.146	0.290	−0.384	−0.091	0.078	–	0.014	0.207
DR										
CL	−0.354	0.231	−0.076	0.257	−0.697*	−0.016	−0.138	–	0.016	−0.197
SO	0.068	0.021	0.033	−0.002	−0.482	−0.536	−0.087	−0.571	0.091	0.063
HM										
CL	0.565	0.029	0.220	−0.172	−0.292	0.273	0.161	–	0.333	0.043
SO	0.140	0.094	0.136	−0.054	−0.419	0.146	0.056	−0.634	−0.366	0.067
PS										
CL	0.068	0.138	0.023	0.062	−0.513	−0.085	−0.165	−1.000	0.172	−0.119
SO	−0.058	−0.020	−0.280	−0.069	−0.282	−0.038	−0.169	−0.357	−0.031	−0.026
GV										
CL	0.061	0.062	0.014	−0.257	−0.654**	0.004	−0.072	0.014	−0.245	−0.143
SO	−0.071	0.073	−0.117	−0.313	−0.639**	0.025	−0.101	−0.105	0.086	−0.167
NC										
CL	0.103	0.069	–	−0.451	−0.666**	0.049	−0.458	–	0.231	0.053
SO	0.008	−0.097	−0.064	−0.210	−0.478**	−0.445	0.078	−0.226	0.096	0.055
PC										
CL	0.014	0.045	0.014	0.182	−0.374	−0.071	−0.311	–	0.143	−0.053
SO	0.112	0.212	0.114	0.144	−0.638**	−0.225	−0.037	0.039	0.110	−0.119
RP										
CL	0.042	–	–	−0.242	−0.629**	−0.110	−0.022	–	−0.322	0.140
SO	0.082	0.173	−0.101	0.014	−0.376	−0.043	−0.110	−0.360	0.042	0.046
AB										
CL	–	0.037	–	0.208	−0.205	0.225	0.151	–	0.094	0.036
SO	0.082	0.039	−0.072	−0.144	−0.507**	−0.517	0.085	0.098	0.042	0.150
CC										
CL	0.013	–	–	0.285	−0.479**	−0.056	−0.057	–	−0.159	0.020
SO	0.070	−0.134	0.139	−0.171	−0.626**	−0.284	−0.020	−0.491	−0.403	−0.062
PL										
CL	0.038	–	–	−0.005	−0.343	0.033	0.014	0.016	0.049	−0.654**
SO	0.128	0.176	0.128	−0.081	−0.441**	0.160	0.149	−0.167	0.042	0.019
PB										
CL	−0.617	–	0.059	0.059	1.000	0.029	−0.133	–	–	–
SO	−0.109	−0.029	0.123	0.043	−0.612**	−0.423	−0.189	0.098	−0.184	−0.026

Allelic frequencies at the other loci also differed between forms at one or more sites, with the exceptions of *PEP-2* and *PGM*, whose frequencies did not vary among sites or forms (Table 1). All the alleles found in the clonal form of *A. elegantissima* were also present in the solitary form. Populations of the solitary form did, however carry, at low frequency, several unique alleles that never occurred in clonal individuals (Table 1).

The mean number of alleles and mean heterozygosity per locus (observed and expected) were greater for the solitary form at all sites (Table 3).The mean number of alleles per locus ranged from only 2.0 to 2.9 in clonal

Table 3 *Anthopleura elegantissima*. Mean number of alleles per locus and observed and expected mean heterozygosity values for clonal (*CL*) and solitary (*SO*) populations [H_o observed heterozygosity; H_e Hardy–Weinberg expected heterozygosity (Nei's 1978 unbiased estimate)]. Standard deviations in parentheses (*Site abbreviations* as in Fig. 1)

Site, form	Mean No. alleles per locus	Mean H_o	Mean H_e
SH			
CL	2.7 (0.4)	0.290 (0.062)	0.318 (0.073)
CB			
CL	2.9 (0.4)	0.357 (0.088)	0.362 (0.084)
DR			
CL	2.7 (0.3)	0.306 (0.063)	0.372 (0.073)
SO	3.9 (0.6)	0.374 (0.075)	0.433 (0.071)
HM			
CL	2.4 (0.2)	0.365 (0.076)	0.336 (0.068)
SO	4.0 (0.7)	0.398 (0.081)	0.422 (0.076)
PS			
CL	2.7 (0.2)	0.258 (0.057)	0.314 (0.077)
SO	4.1 (0.7)	0.401 (0.066)	0.464 (0.069)
GV			
CL	2.6 (0.3)	0.216 (0.058)	0.280 (0.074)
SO	3.9 (0.5)	0.359 (0.049)	0.450 (0.069)
NC			
CL	2.3 (0.3)	0.210 (0.056)	0.285 (0.077)
SO	3.9 (0.7)	0.390 (0.073)	0.454 (0.070)
PC			
CL	2.7 (0.4)	0.228 (0.060)	0.272 (0.083)
SO	3.9 (0.6)	0.403 (0.072)	0.443 (0.077)
RP			
CL	2.1 (0.3)	0.195 (0.061)	0.258 (0.080)
SO	4.1 (0.7)	0.419 (0.076)	0.448 (0.076)
AB			
CL	2.0 (0.3)	0.249 (0.088)	0.236 (0.084)
SO	4.0 (0.6)	0.421 (0.083)	0.470 (0.076)
CC			
CL	2.3 (0.3)	0.220 (0.078)	0.252 (0.089)
SO	3.8 (0.6)	0.382 (0.056)	0.487 (0.052)
PL			
CL	2.6 (0.3)	0.234 (0.081)	0.264 (0.089)
SO	3.6 (0.6)	0.396 (0.077)	0.406 (0.077)
PB			
CL	2.0 (0.3)	0.244 (0.110)	0.229 (0.084)
SO	4.3 (0.7)	0.378 (0.071)	0.454 (0.074)

forms, but from 3.6 to 4.3 in solitary forms (Table 3). Among clonal forms, the mean observed heterozygosity per locus ranged from 0.195 to 0.365; in solitary forms, it ranged from 0.359 to 0.421 (Table 3). This comparison, however, should be interpreted cautiously: because we eliminated any duplicate samples of the same multilocus genotypes at any given site, the sample size for clonal forms was generally smaller than that for solitary forms (Table 1).

In terms of genetic distances, populations of clonal individuals from sites separated by >1000 km were more similar to one another than they were to solitary individuals from the same site (Table 4). The mean pairwise genetic distance between clonal and solitary forms from the same site was 0.105 (SD = 0.019, $n = 11$), compared to mean pairwise distances of 0.043 (SD = 0.024, $n = 78$) among the 13 populations of the clonal form and 0.020 (SD = 0.007, $n = 55$) among the 11 populations of the solitary form.

The neighbor-joining tree based on pairwise Cavalli-Sforza chord distances between populations unambiguously shows that the clonal and solitary forms of *Anthopleura elegantissima* are distinct taxa, with the clonal forms falling into a monophyletic clade supported by a bootstrap value of 98% (Fig. 2). The phylogenetic relationship between clonal and solitary forms is poorly resolved within this tree, however, with little support for the node connecting the clonal clade to populations of the solitary form. Likewise, there is little support for most of the nodes within both the clonal and solitary clades, making the genetic relationships among the different populations of each form uncertain. Three nodes do, however, have moderate support (bootstrap values >60%): (1) populations of the clonal form found south of San Francisco Bay form a clade separate from the three northernmost clonal populations sampled; (2) populations of the solitary form from Hopkins Marine Station (HM-SO) and Point Sal (PS-SO) are genetically distinct and lie outside the clade containing the rest of the solitary populations; (3) populations of the solitary form from Resort Point (RP-SO) and Point Loma (PL-SO) form a clade distinct from the remaining solitary populations (Fig. 2). In general, however, there appears to be little recognizable pattern in geographic structure among populations of either form.

Genetic structure within and among populations of clonal and solitary forms

Because the heterogeneity chi-square reveals significant differences in allele frequencies at some loci (Table 1), and the neighbor-joining tree (Fig. 2) suggests that clonal and solitary forms represent distinct taxa, we analyzed population genetic structure separately for the two forms of *Anthopleura elegantissima*. Estimates of *f* (the inbreeding coefficient) (Table 5) reflect the anomalous genotype frequencies we found at the *PEP-2* locus (Table 2). When *PEP-2* is included in the analysis, mean

Table 4 *Anthopleura elegantissima*. Matrix of genetic distances using Cavalli-Sforza chord distance (*CL* clonal; *SO* solitary; *site abbreviations* as in Fig. 1)

	SH CL	CB CL	DR CL	DR SO	HM CL	HM SO	PS CL	PS SO	GV CL	GV SO	NC CL	NC SO	PC CL	PC SO	RP CL	RP SO	AB CL	AB SO	CC CL	CC SO	PL CL	PL SO	PB CL	PB SO
SH CL		0.017	0.021	0.090	0.067	0.076	0.032	0.076	0.050	0.083	0.054	0.081	0.046	0.081	0.052	0.079	0.070	0.093	0.058	0.083	0.063	0.095	0.069	0.070
CB CL			0.026	0.071	0.084	0.075	0.042	0.075	0.065	0.075	0.073	0.072	0.056	0.067	0.070	0.070	0.074	0.085	0.067	0.072	0.072	0.097	0.084	0.064
DR CL				0.085	0.051	0.072	0.036	0.072	0.059	0.072	0.058	0.074	0.050	0.075	0.061	0.072	0.073	0.088	0.063	0.073	0.066	0.089	0.088	0.072
DR SO					0.120	0.021	0.096	0.021	0.110	0.021	0.113	0.015	0.105	0.012	0.119	0.014	0.122	0.029	0.111	0.018	0.103	0.029	0.159	0.026
HM CL						0.102	0.041	0.088	0.041	0.096	0.037	0.100	0.043	0.111	0.037	0.110	0.051	0.117	0.047	0.116	0.048	0.100	0.099	0.108
HM SO							0.088	0.009	0.105	0.009	0.109	0.013	0.103	0.019	0.115	0.014	0.126	0.026	0.112	0.018	0.102	0.028	0.167	0.020
PS CL								0.081	0.020	0.083	0.028	0.083	0.019	0.089	0.019	0.094	0.028	0.096	0.030	0.088	0.037	0.088	0.067	0.088
PS SO									0.098	0.010	0.108	0.010	0.097	0.014	0.110	0.016	0.120	0.023	0.107	0.015	0.096	0.027	0.161	0.018
GV CL										0.092	0.017	0.092	0.010	0.103	0.012	0.109	0.018	0.088	0.020	0.103	0.019	0.102	0.070	0.097
GV SO											0.097	0.010	0.090	0.010	0.110	0.012	0.112	0.019	0.101	0.014	0.086	0.019	0.152	0.018
NC CL												0.106	0.013	0.106	0.021	0.107	0.020	0.104	0.017	0.109	0.017	0.099	0.057	0.107
NC SO													0.098	0.013	0.116	0.010	0.122	0.026	0.108	0.012	0.096	0.024	0.144	0.017
PC CL														0.100	0.014	0.100	0.012	0.093	0.012	0.101	0.013	0.101	0.065	0.095
PC SO															0.119	0.010	0.125	0.025	0.111	0.011	0.099	0.026	0.151	0.025
RP CL																0.112	0.013	0.112	0.011	0.119	0.021	0.111	0.069	0.108
RP SO																	0.115	0.022	0.109	0.022	0.094	0.044	0.167	0.017
AB CL																		0.126	0.008	0.119	0.017	0.122	0.076	0.108
AB SO																			0.113	0.022	0.097	0.029	0.145	0.022
CC CL																				0.107	0.007	0.107	0.058	0.103
CC SO																					0.102	0.038	0.168	0.021
PL CL																						0.093	0.050	0.107
PL SO																							0.167	0.038
PB CL																								0.150
PB SO																								

SH: Strawberry Hill, OR
CB: Cape Blanco, OR
DR: Doran Rocks, CA
HM: Hopkins Marine Station, CA
PS: Point Sal, CA
GV: Gaviota Beach, CA
NC: Nicholas Canyon, CA

PC: Paradise Cove, CA
RP: Resort Point, CA
AB: Aliso Beach, CA
CC: Casa Cove, CA
PL: Point Loma, CA
PB: Punta Banda, BC

Fig. 2 *Anthopleura elegantissima.* Strict consensus, majority rule neighbor-joining tree, based on Cavalli-Sforza chord distances, depicting genetic relationships among clonal (*CL*) and solitary (*SO*) forms [*Numbers at nodes* are bootstrap percentages based on 200 iterations (only values >50% shown)]

values of *f*, estimated by jackknifing over populations, are 0.134 and 0.125 for clonal and solitary forms, respectively. Without the *PEP-2* locus, the mean values of *f* averaged over the remaining nine loci drop to 0.036 (not significantly different from 0) for the clonal form and 0.060 for the solitary form; *F* (total inbreeding) correspondingly decreases (Table 5). The removal of any of the other loci does not significantly change estimates of overall mean *f* and *F*. These low values of *f* suggest that both clonal and solitary forms of *A. elegantissima* experience little if any inbreeding.

Values of θ (the fixation index) do, however, differ significantly between the clonal and solitary forms of *Anthopleura elegantissima*: populations of the clonal

form exhibit a small but significant degree of genetic differentiation ($\theta = 0.061$, $p < 0.05$), whereas populations of the solitary form do not ($\theta = 0.007$, NS) (Table 5). The 95% confidence limits of these θ values are non-overlapping, indicating a significant difference between the two forms (Table 5). The higher θ recorded for the clonal form is not an artifact of the wider geographic range over which it was sampled: when the two Oregon populations were removed from the analysis, θ did decrease ($\theta = 0.044$, $p < 0.05$), but remained significantly greater than the value for the solitary form.

Discussion

Taxonomic status of clonal and solitary forms
of *Anthopleura elegantissima*

Our neighbor-joining tree (Fig. 2) and the significant differences in allele frequencies between forms at every site we sampled (Table 1) suggest that the clonal and solitary forms of *Anthopleura elegantissima* are strongly differentiated genetically across their entire range of sympatry. Although it is possible that we may have misclassified some individuals as either clonal or solitary forms, such errors would obscure rather than enhance the patterns of differentiation that we detected. These genetic data closely agree with Francis' (1979) analysis of phenotypic variation in eight populations spanning a similar geographical range: she found that differences in the frequencies of color pattern polymorphisms were much greater between clonal and solitary *A. elegantissima* within each site than between geographically distant populations of each form. Thus, in contrast to Smith and Potts (1987), we conclude that Francis' designation of the two forms as nominal species can be justified by genetic, as well as morphological, ecological, and life-history criteria. We therefore propose that the two forms be recognized as distinct species, despite the fact that they both lack fixed, diagnostic alleles. Because Hand's (1955) description of *A. elegantissima* ("the aggregating anemone") clearly applies to the clonal form, as he describes the process of longitudinal fission and states that this species is typically found in extensive aggregations on very exposed rock surfaces, we suggest that the clonal form retain this binomial, and the solitary form be described as a new species.

Although both the allelic and color-pattern frequencies (Francis 1979) strongly suggest reproductive isolation of the two forms, neither the reproductive phenology of the solitary form nor the potential for cross-fertilization to occur among the two forms have, to our knowledge, been studied. The two forms probably do reproduce at similar times however, as they have been observed to spawn synchronously in mid-June after being maintained for over a year in an outdoor seawater tank (J.S. Pearse personal communication). Direct evidence of barriers to inter-form hybridization would further confirm the reproductive isolation of the two

Table 5 *Anthopleura elegantissima*. Values of *F*, *f* and θ for clonal and solitary populations from NE Pacific coast. Values shown are means for each locus jackknifed over populations (standard errors in parentheses). Overall means were determined by jackknifing over all loci and populations. Upper and lower 95% confidence intervals (*CI*) of overall means determined by bootstrapping over loci and populations

Locus	Clonal			Solitary		
	f	*F*	θ	*f*	*F*	θ
MDH-1	−0.034 (0.170)	0.077 (0.101)	0.117 (0.077)	−0.032 (0.024)	−0.028 (0.025)	0.004 (0.005)
MDH-2	0.022 (0.085)	0.093 (0.089)	0.072 (0.020)	−0.035 (0.034)	−0.043 (0.033)	−0.008 (0.002)
6PGD	−0.042 (0.060)	0.070 (0.051)	0.107 (0.025)	0.009 (0.040)	0.001 (0.041)	−0.009 (0.001)
HK	−0.055 (0.078)	−0.020 (0.075)	0.033 (0.015)	0.091 (0.037)	0.093 (0.035)	0.003 (0.006)
PEP-2	0.454 (0.051)	0.474 (0.045)	0.037 (0.020)	0.511 (0.035)	0.520 (0.034)	0.017 (0.010)
PGI-1	0.014 (0.028)	0.081 (0.033)	0.068 (0.021)	0.237 (0.076)	0.237 (0.076)	−0.001 (0.004)
PGM	0.099 (0.046)	0.117 (0.047)	0.020 (0.010)	0.044 (0.035)	0.049 (0.036)	0.005 (0.005)
IDH-1	0.656 (0.529)	0.651 (0.530)	−0.008 (0.004)	0.279 (0.077)	0.286 (0.077)	0.010 (0.009)
IDH-2	−0.013 (0.079)	0.022 (0.072)	0.035 (0.017)	0.113 (0.095)	0.223 (0.172)	0.115 (0.091)
MPI	0.084 (0.076)	0.228 (0.085)	0.156 (0.040)	0.012 (0.028)	0.013 (0.028)	0.001 (0.003)
Mean	0.134 (0.093)	0.182 (0.084)	0.056 (0.016)	0.125 (0.069)	0.132 (0.070)	0.007 (0.005)
[*CI*]	[−0.014–0.273]	[0.047–0.315]	[0.035–0.094]	[0.026–0.278]	[0.030–0.290]	[0.000–0.022]
Mean without *PEP-2*	0.036 (0.029)	0.095 (0.027)	0.061 (0.021)	0.060 (0.028)	0.066 (0.029)	0.007 (0.006)
[*CI*]	[−0.023–0.076]	[0.036–0.140]	[0.033–0.108]	[0.023–0.125]	[0.025–0.137]	[−0.001–0.023]

forms suggested by our allozyme data. The opposite result – demonstration of ability to hybridize the two forms – would not, however, be sufficient to negate our conclusions regarding their status as genetically distinct species, because hybridization among genetically and morphologically distinct species occurs naturally in many diverse taxa (Templeton 1989).

There are several factors that could explain the discrepancies between our results and those of Smith and Potts (1987), who concluded that clonal and solitary forms of *Anthopleura elegantissima* could not be genetically distinguished on the basis of pairwise genetic distance values (Nei's *D*). In reaching this conclusion, they first estimated genetic distances between populations of each form, and asserted that intra-form values of Nei's *D* did not differ significantly from 0 [although pairwise genetic distances between clonal populations were generally higher than those between solitary populations (see their Table 3)]. They used this result to justify pooling all populations of each form before calculating a single estimate of genetic distance between the clonal and solitary forms (their Table 4). Because this single inter-form distance value was "very similar to the mean intra-form genetic distances (Smith and Potts 1987: p. 541)," they concluded that there was no significant genetic differentiation between clonal and solitary forms. Statistically, this is a dubious procedure, because the strongest rationale for pooling would require that neither allelic nor genotypic frequencies differ significantly among sites within forms (Weir 1996). Likewise, the strongest evidence for lack of genetic differentiation among forms would be no difference in allelic frequencies between forms within each site. Smith and Potts did not, however, provide allele frequency data for populations of clonal and solitary forms.

The very high F_{IS} values and significant heterozygote deficiencies Smith and Potts (1987) found for both forms of *Anthopleura elegantissima* are also at odds with our results. Several of the loci they included in their study are ones we found difficult to score consistently, and for which we suspected null alleles existed (e.g. *LAP*), or for which we were unable to obtain activity reliably (e.g. *XDH*). It is possible that the presence of unrecognized null alleles could have contributed to the large heterozygote deficits and departures from Hardy–Weinberg expectations they observed. Nonetheless, several of the loci for which they recorded significant heterozygote deficits are ones for which we did not detect any evidence of null alleles. Finally, it is possible that Smith and Potts misidentified forms, which could explain both the lack of differences in allele frequencies and the heterozygote deficits (due to a Wahlund effect) they found in both forms.

Population genetics of *Anthopleura elegantissima* and *Anthopleura* sp.

Because both *Anthopleura elegantissima* and *Anthopleura* sp. freely spawn gametes and possess long-lived planktonic larvae (Ford 1964; Siebert 1974; Jennison 1979), we expected – assuming evolutionary equilibrium – that neither species would exhibit deviations from panmixia or substantial genetic structure within populations beyond that produced by association of clonemates. Indeed, only 13 of 217 analyses revealed significant heterozygote deficiencies, and once we excluded *PEP-2* from the analysis, we found only a single significant deviation from Hardy–Weinberg expectations. Likewise, in *A. elegantissima* the inbreeding coefficient, *f*, does not

differ significantly from 0, whereas in *Anthopleura* sp., *f* is small, although significantly greater than 0 (based on confidence intervals bootstrapped across populations and loci, excluding *PEP-2*).

The neighbor-joining tree, based on genetic distances, reveals little genetic differentiation among populations of either species. In terms of θ, the fixation index, *Anthopleura* sp. shows no signs of population subdivision; however, *A. elegantissima* does exhibit a small, but significant value of θ, comparable to several other broadcasting species of clonal benthic invertebrates (Stoddart 1984; Ayre et al. 1991; Benzie et al. 1995; Burnett et al. 1995). Our estimate of θ = 0.061 (95% confidence interval, CI: 0.033 to 0.108) is not significantly different from that determined by Edmands and Potts [1997; θ = 0.141 (95% CI: 0.094 to 0.180)] for five populations of *A. elegantissima* collected over a broader geographic range (northern Washington to southern California).

These species-specific differences in population structure probably result from a combination of the distinct reproductive modes and evolutionary histories of the clonal *Anthopleura elegantissima* compared to the solitary *Anthopleura* sp. All else being equal, species that propagate clonally should have a smaller effective population size (N_e) than species which only reproduce sexually (Wright 1978; Ayre and Dufty 1994). This effect of cloning on the size of breeding populations could arise from some combination of the reduced number of genets relative to ramets in a population of fixed number (Hughes et al. 1992; Knowlton and Jackson 1993; Ayre and Dufty 1994), the potential for enormous variance among genets in sexual reproductive output (Hughes 1989; Babcock 1991; Brazeau and Lasker 1992; Hughes et al. 1992), extremely long generation times (which will retard the approach to equilibrium: Potts 1984; Hughes et al. 1992), and overlapping generations (Potts 1984; Hughes et al. 1992). This, in turn, should increase the rate at which genetic drift causes differentiation among populations of clonal taxa relative to populations of exclusively sexual taxa (Hughes et al. 1992). The effect of cloning on the number of alleles per locus is more difficult to assess, and strongly depends on the extinction/recolonization dynamics of individual populations (Slatkin 1977; Wade and McCauley 1988; Whitlock and McCauley 1990; Whitlock 1992; Dybdahl 1994; Ruckelshaus 1994).

The observed species-specific differences in population structure could also arise if *Anthopleura elegantissima* were evolutionarily derived from *Anthopleura* sp. If this is the case (as discussed in the following subsection), and *A. elegantissima* were only recently derived, then insufficient time may have passed since the evolution of reproductive isolation for the clonal species to have reached genetic equilibrium. This alone may lead to transiently higher levels of population subdivision (Slatkin 1977; Wade and McCauley 1988; Whitlock and McCauley 1990; Whitlock 1992; Dybdahl 1994; Hellberg 1994; Ruckelshaus 1994). The approach to genetic equilibrium may be further retarded in clonal taxa by

their characteristically long generation times (Potts 1984). Evolutionary disequilibrium could also account for the observed species-specific differences in allelic diversity.

Evolutionary relationships between *Anthopleura elegantissima* and *Anthopleura* sp.

Populations of *Anthopleura elegantissima* (the clonal taxon) are clearly monophyletic (Fig. 2), implying that clonal reproduction has evolved only once within this lineage. Although the neighbor-joining tree does not provide unequivocal evidence that *A. elegantissima* arose from a solitary ancestor, the allelic differences between *A. elegantissima* and *Anthopleura* sp. support such a scenario. Populations of *A. elegantissima* harbor a subset of the same alleles found in *Anthopleura* sp., suggesting that some alleles present in ancestral solitary forms were lost during speciation, but that not enough time has passed since this event for new, unique alleles to have arisen in *A. elegantissima*. Similarly, there may have been insufficient time since speciation to permit the fixation of unique alleles in the putatively ancestral *Anthopleura* sp.

Smith and Potts' (1987) UPGMA dendrogram of Nei's genetic distances among the four North American species of *Anthopleura* suggests that *A. artemisia* and the solitary *A. xanthogrammica* (Hand 1955) are ancestral to both *A. elegantissima* and *Anthopleura* sp. On the basis of what appeared to be fission scars, Hand reported that *A. artemisia* reproduced clonally by longitudinal fission, but this mode of reproduction has never subsequently been verified in this species. Lack of additional evidence of clonality, coupled with the typical burrowing and hole-dwelling habits of this species, have led several authors to suggest that *A. artemisia* does not reproduce clonally (Francis 1973b; D. Fautin personal communication). If this is true, then Smith and Potts' dendrogram also supports the evolution of the clonal *A. elegantissima* from a solitary ancestor. To determine unequivocally that cloning is a derived character state will require a complete phylogenetic and reproductive analysis of the genus *Anthopleura*.

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