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Population genetic structure in brooding sea anemones (*Epiactis* spp.) with contrasting reproductive modes

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Abstract Effects of dispersal and mating systems on the genetic structure of populations were evaluated by comparing five sea anemones: four *Epiactis* species that brood their offspring to the juvenile stage and one *Anthopleura* species that broadcasts gametes and has pelagic, planktotrophic larvae. The anemones were sampled at sites ranging from British Columbia to southern California between 1988 and 1992 and were analyzed by enzyme electrophoresis and by multilocus DNA-fingerprinting. Results were only partially consistent with expectations. While all four brooding species had lower observed heterozygosities than the broadcasting species, not all brooding species had greater population subdivision than the broadcasting species. The self-fertile *E. prolifera* had the expected evidence of intense local inbreeding (f = 0.955); unexpectedly, the cross-fertile *E. lisbethae* and *E. ritteri* also had similar departures from random mating (f = 0.957 and 0.831, respectively) probably due to biparental inbreeding among near neighbors in small, highly subdivided populations.

Introduction

The genetic structure of marine populations should reflect both larval dispersal potential and mating systems. Larval dispersal capacity ranges from a maximum in species whose larvae spend many months in the plankton (e.g. Scheltema 1971) to a minimum in species which brood their larvae to the juvenile stage (e.g. Dunn et al. 1980). Reduced larval duration is often correlated with less genetic variation within populations and greater differentiation between populations (Scheltema 1971, 1978). Many empirical studies support these predictions (e.g. Berger 1973; Duffy 1993; Hunt 1993; Russo et al. 1994), but there are also many exceptions (see Burton 1983; Hedgecock 1986; Palumbi 1994).

The effects of mating systems on population genetic structure are also difficult to predict, due to numerous confounding factors. In species with moderate larval dispersal potential, the following genetic patterns are usually expected (e.g. Hunt and Ayre 1989): outcrossing should maintain high genotypic diversity and Hardy–Weinberg equilibria within populations, with low levels of differentiation among populations; self-fertilization, as the most extreme form of inbreeding, should cause severe heterozygote deficits and low genetic variation within populations, with substantial divergence among populations; and asexual reproduction should result in low genotypic diversity and departures from random mating within populations, with high levels of differentiation among populations.

While many outcrossing species fit these predictions (e.g. Hunt and Ayre 1989; Russo et al. 1994), there are also numerous reports of such species having surprisingly low genetic variation (e.g. the marine snail *Littorina saxatilis*: Knight et al. 1987), large departures from random mating (e.g. the ascidian *Botryllus schlosseri*: Grosberg 1991) and striking differentiation among populations (e.g. the copepod *Tigriopus californicus*: Burton and Feldman 1981). There have been few studies of the genetic effects of self-fertilization in marine systems, but several species capable of self-fertilization do not have the expected population genetic effects of inbreeding, apparently because selfing is rare in the field (the coral *Goniastrea favulus*: Stoddart et al. 1988; the ascidians *Corella inflata* and *Chelyosoma productum*: Cohen 1990; the bryozoan *Celleporella hyalina*: Hunter

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and Hughes 1993). Consequences of self-fertilization are much better understood in terrestrial plants, where numerous studies have documented the expected inter-population differentiation and low intra-population heterozygosity (Layton and Ganders 1984; Hamrick and Godt 1989; Heywood 1991). The effects of asexual reproduction have been studied intensively in marine invertebrates (e.g. Shick and Lamb 1977; Ayre 1984, 1985; Hoffmann 1986; Smith and Potts 1987; Ayre et al. 1991; McFadden and Aydin 1996). Compared with strictly sexual species, asexually reproducing species generally have reduced genotypic diversity (possibly due to founder-effects: Hoffmann 1986), increased population differentiation (possibly due to local adaptation: Ayre 1985), and deviations from Hardy–Weinberg equilibria including both heterozygote excesses and deficits (presumably due to the random asexual proliferation of genotypes: Hoffmann 1986).

When observed genetic structures of populations do not match expectations based on larval dispersal potential and mating systems, many confounding factors may be responsible. For example, gene flow between populations may be increased by rafting of adults (Jokiel 1984; Highsmith 1985), decreased by restricted gamete dispersal (Grosberg 1991), or decreased by larval behavior favoring local recruitment (Burton and Feldman 1981; Grosberg and Quinn 1986; Knowlton and Keller 1986). Similarly, genetic variation can be strongly influenced by effective population size (Chakraborty and Nei 1977; Knight et al. 1987), natural selection (Koehn et al. 1976; Ayre 1985) and historical effects influencing local extinction or recolonization rates (Hellberg 1994). Interactions between effects of dispersal and effects of mating systems may be difficult to disentangle in certain situations, such as for organisms with extremely restricted dispersal where the effects of biparental inbreeding may mimic the effects of self-fertilization (Ennos and Clegg 1982).

In this study, we assess the genetic effects of larval dispersal and mating systems in four brooding sea anemone species in the genus *Epiactis* Verrill, 1869 and one broadcasting sea anemone species, *Anthopleura elegantissima* Brandt, 1835. The genus *Epiactis* provides an opportunity to test the genetic consequences of variation in brooding site and in potential for self-fertilization. All four species on the Pacific coast of North America are morphologically similar, brood their young to a benthic juvenile stage, and are not known to reproduce by budding or fission. However, each species has a different combination of internal vs external brooding and hermaphroditism vs gonochory (Fautin and Chia 1986; Edmands 1996) (present Table 1). *E. prolifera* Verrill, 1869, is an externally brooding, continually reproducing, gynodioecious hermaphrodite (first female, then hermaphroditic) which is abundant from Alaska (Dunn 1975a,b; 1977a,b) to Baja California (Edmands personal observation). *E. lisbethae* Fautin and Chia, 1986, is an externally brooding, seasonally reproducing gonochore known from British Columbia (Fautin and Chia 1986) to central California (Edmands 1995). *E. ritteri* Torrey, 1902, is an internally brooding gonochore found from Alaska to central California (Hand and Dunn 1974). *E. fernaldi* Fautin and Chia, 1986, is an internally brooding hermaphrodite known only from Barkley Sound, British Columbia, and San Juan Island, Washington (Fautin and Chia 1986; Fautin personal communication). Mature offspring in the two external brooders ''crawl'' or ''glide'' directly onto the surrounding substrate (MacGinitie and MacGinitie 1968; Ricketts and Calvin 1968; Edmands 1995). Release of offspring in the two internal brooders has not been described, but might be expected to result in somewhat greater dispersal, particularly if release occurs while the sea anemones are submerged. For comparison of the effects of dispersal capacity, genetic variation was also assessed in the clonal form of the broadcasting species *A. elegantissima*, a gonochore with external fertilization and planktotrophic larvae (Siebert 1974; Jennison 1979), which has a range from Alaska to Baja California (Morris et al. 1980).

Breeding systems in the genus *Epiactis* have been determined by direct genetic comparisons of adults and their brooded offspring (Table 1). Electrophoretic studies by Bucklin et al. (1984) demonstrated that

Table 1 *Epiactis* spp. and *Anthopleura elegantissima*. Summary of life-history characteristics of five species studied (*nd* no data available). See ''Introduction'' for further information and references

Species	Offspring	Sex expression	Timing of reproduction	Mating system
E. prolifera	brooded externally	gynodioecious hermaphrodite	continuous	hermaphrodites self-fertile; females either parthenogenetic or cross-fertile
E. lisbethae	brooded externally	gonochore	seasonal	cross-fertile
E. ritteri	brooded internally	gonochore	nd	cross-fertile
E. fernaldi	brooded internally	simultaneous hermaphrodite	nd	nd
A. elegantissima	planktonic larvae	gonochore	seasonal	clonal and cross-fertile

E. prolifera is capable of self-fertilization; each of 22 homozygous adults tested had 100% identical progeny, while the three heterozygous adults had offspring whose allelic frequencies were consistent with the 1:2:1 ratios expected from self-fertilization. However, self-fertilization cannot explain the origin of offspring on smaller, strictly female adults (Dunn 1975b). Allozyme studies of the same species by Edmands (1995) found only homozygous brooding adults, and all offspring were identical to their parent (even for rare homozygotes). Multilocus DNA fingerprints, which often reveal greater genetic variation than allozymes but do not allow heterozygotes to be identified (Jeffreys et al. 1985a,b; Lynch 1988, 1990, 1991), showed that both hermaphroditic and strictly female parents had offspring that were genetically indistinguishable from the adult (Edmands 1995). These results are consistent with Bucklin et al.'s (1984) report of self-fertilization in hermaphrodites, and suggest that females are reproducing either by ameiotic parthenogenesis or by cross-fertilization with genetically similar neighbors. In contrast, DNA fingerprints of both *E. lisbethae* and *E. ritteri* had bands in offspring that were not present in their mothers, suggesting that these species are at least facultative outcrossers (Edmands 1995). The breeding system in *E. fernaldi* remains unknown but, because it is a simultaneous hermaphrodite, it is potentially capable of self-fertilization. Finally, *Anthopleura elegantissima* is known to reproduce asexually by binary fission and sexually by externally-fertilized planktonic larvae (Hand 1955; Francis 1973; Jennison 1979).

We hypothesized (1) that all four brooding *Epiactis* species should have less genetic variation within populations and greater differentiation among populations than the broadcaster *Anthopleura elegantissima*; and (2) that the selfing *E. prolifera* should have lower within-population variation, higher local inbreeding, and greater population differentiation than the outcrossing *E. lisbethae* and *E. ritteri.* We determined genetic structure by enzyme electrophoresis and by multilocus DNA-fingerprinting. Enzyme electrophoresis is generally highly informative for comparisons of conspecific populations (Hillis and Moritz 1990), and the data are readily analyzed by established statistical methods. DNA-fingerprinting should reveal substantially greater variation than enzyme electrophoresis because of extremely high mutation rates at minisatellite loci (Jeffreys et al. 1985a,b; 1988), but the data are more difficult to interpret. Genetic interpretation of multilocus DNA fingerprints is complicated by not knowing which bands belong to which loci; however, methods have been developed to infer genetic data from DNA fingerprint phenotypes (Lynch 1988, 1990, 1991). Multilocus DNA-fingerprinting is typically used for comparisons at the individual level (e.g. Burke and Bruford 1987; Wetton et al. 1987), but it has also proven useful for population comparisons, particularly in species with low levels of genetic variation (Reeve et al. 1990; Rogstad et al. 1991; Coffroth et al. 1992; Zeh et al. 1992).

Materials and methods

Collections

Specimens of *Epiactis* spp. and *Anthopleura elegantissima* were collected between December 1988 and July 1992 from intertidal and shallow subtidal habitats from British Columbia to southern California (Table 2; Fig. 1). Sample sizes were limited by the scarcity of some species, and ranged from 8 to 30 individuals per species per site (defined as a single rocky outcrop). Whenever possible, individuals were taken from at least 1 m apart to reduce chances of resampling the same clone if asexual reproduction should occur. Anemones were maintained in flowing seawater tables at the Long Marine Laboratory, Santa Cruz, California, and were starved for at least 3 d before they were used for genetic analysis, or were frozen and stored at −70 °C.

Table 2 *Epiactis* spp. and *Anthopleura elegantissima.* Collection sites, habitats and dates. Sites are listed from north to south and include both intertidal (*i*) and subtidal (*s*) habitats (*BC* British Columbia; *WA* Washington; *OR* Oregon; *CA* California; *I* Island; *Pt* Point)

Species, Site No. and Location	Habitat	Dates (mo/yr)
Epiactis prolifera		
2. Neck Pt, Shaw I, WA	s	7/90, 6/91
3. Brown I, WA	s	6/90, 6/91
5. Mar Vista, San Juan I, WA	i	6/90, 6/91
8. Tatoosh I, WA	i	7/92[a]
9. Cape Arago, OR	i	7/90, 5/91, 5/92
10. Bodega Bay, CA	i	3-4/90, 5/91, 5/92
11. Pigeon Point, CA	i	6/89, 10/89, 4/90, 10-12/91, 3-4/91, 8/91, 6/92
12. Ship Rock, Santa Catalina I, CA	s	3-5/89, 4/90
13. Bird Rock, Santa Catalina I, CA	s	5/89, 4/90
Epiactis lisbethae		
5. Mar Vista, San Juan I, WA	i	6/90, 6/91
9. Cape Arago, OR	i	7/90, 5/91, 5/92
11. Pigeon Pt, CA	i	5/90
Epiactis ritteri		
1. Execution Rocks, Vancouver I, BC	i	7/90
8. Tatoosh I, WA	i	7/92[a]
10. Bodega Bay, CA	i	12/88[b] , 4/90, 5/91, 5/92, 7/92
Epiactis fernaldi		
4. MacGinitie Cave, San Juan I, WA	i	7/90, 6/91, 11/91[c]
7. South Beach, San Juan I, WA	i	7/90, 6/91
Anthopleura elegantissima		
5. Mar Vista, San Juan I, WA	i	7/90
6. Eagle Pt, San Juan I, WA	i	7/90
9. Cape Arago, OR	i	5/91, 5/92
10. Bodega Bay, CA	i	5/91, 5/92
14. Fishermen's Cove, Santa Catalina I, CA	i	5/89

Samples kindly provided by:
[a]RK Grosberg, [b]W Clark, [c]MD McHugh

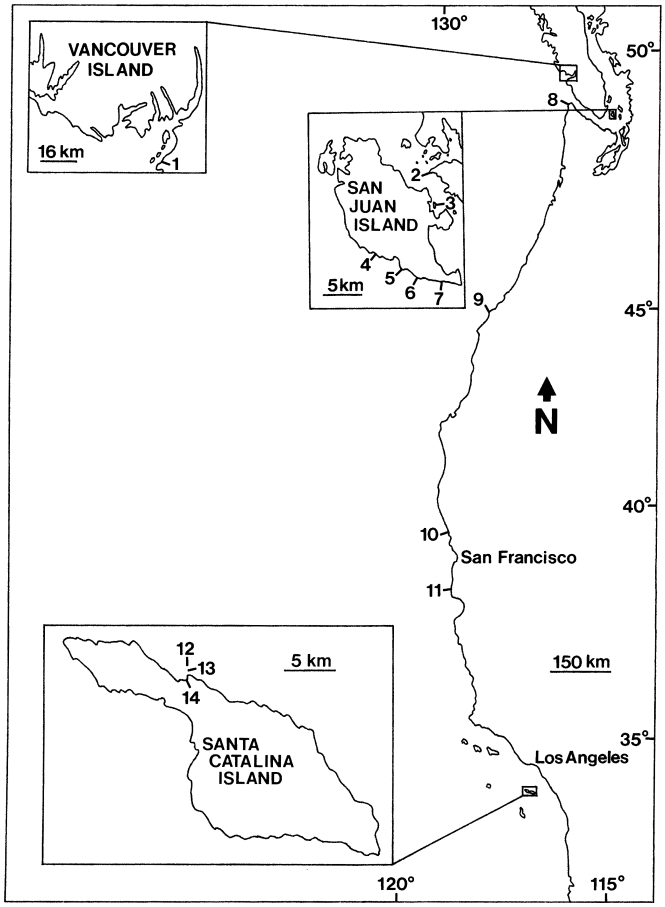

Fig. 1 Collection sites of *Epiactis* spp. and *Anthopleura elegantissima* along Pacific coast of North America (locations numbered as in Table 2)

Allozymes

Horizontal starch-gel electrophoresis followed standard protocols outlined in Aebersold et al. (1987), Murphy et al. (1990) and Edmands (1995). Gels were made of 10.5% starch (Sigma). Three buffer systems (TC1: Ward and Beardmore 1977; TC2: Shaw and Prasad 1970; and LiOH: Shaw and Prasad 1970) were used to resolve 20 enzyme loci (Edmands 1995). Stain recipes were from Murphy et al., except those for CAT and EST-D which were minor modifications of recipes in Aebersold et al. Because *Anthopleura elegantissima* is known to propagate clonally (Hand 1955; Francis 1973), any duplicate 20-locus genotypes were eliminated for this species.

The eletrophoretic data were analyzed using BIOSYS-1 (Version 1.7: Swofford and Selander 1981, 1989) to calculate observed and expected heterozygosities and unbiased genetic distances and genetic identities between all conspecific pairs of populations (Nei 1978). Total inbreeding (F_{IT}) was partitioned into components resulting from inbreeding within subpopulations (F_{IS}) and differentiation among subpopulations (F_{ST}) using estimators described by Weir and Cockerham (1984) ($F = F_{IT}$; $f = F_{IS}$; $\theta = F_{ST}$) The FSTAT program (Goudet 1995) was used to calculate significance of these estimates at each locus by permuting alleles, with mean values and standard errors determined by jackknifing over loci and 95% confidence intervals for the mean values determined by bootstrapping over loci.

DNA-fingerprinting

Methods for DNA-fingerprinting have been described in Edmands (1995). DNA was extracted using CTAB (cetyltrimethyl ammonium bromide) following Coffroth et al. (1992), with modifications described by Edmands (1995). Approximately 10 μg of *Hae*III-digested DNA was loaded into each lane of an agarose gel which was run at 19mA for 36 h in recirculating TBE buffer (0.089 *M* Tris-borate, 0.089 *M* boric acid, 0.002 *M* EDTA) at room temperature. DNA was transferred to nylon membrane (BRL "Photogene") for 36 h using standard capillary transfer. Membranes were probed with either wildtype bacteriophage M13 (Vassart et al. 1987) or with minisatellite probe 33.6 (Jeffreys et al. 1985a,b) and visualized using a chemiluminescent detection system (Tropix). Fingerprint-bands were scored visually by placing the autoradiograph on a lightbox, attaching a sheet of acetate, and tracing bands between 1 and 23 kbases. Lanes were scored for presence/absence of each band. Restriction fragments were scored as the same if they differed by <1 mm in alignment. No attempt was made to compare fragments between gels.

Bandsharing similarities (S) between all possible pairs of individuals were calculated as: $S = 2n_{xy}/(n_x + n_y)$, where n_{xy} is the number of bands shared between Lanes x and y, and n_x and n_y are the total number of bands in Lanes x and y. The complement of this value ($1–S$) is the statistic APD (average percent difference), and can be used as an approximate (downwardly biased) estimate of heterozygosity (Lynch 1990, 1991). Calculating the variance in S is complicated by the fact that the data are not independent, since individuals are used in multiple comparisons (i.e. all pairwise combinations within a gel). We used the software program SIM (Zimmerman 1993) to calculate an unbiased estimate of the true variance (Var) in similarity among individuals (S_x) using the following equation derived by Lynch (1990): $Var(S_x) = 2S_x(1–S_x)(2–S_x)/n(4–S_x)$. The standard error for the S_x values is then calculated as $\sqrt{Var(S_x)}$.

Results

Allozymes

Table 3 shows allele frequencies, mean observed and expected heterozygosities and mean sample sizes for each population of *Epiactis* spp. and *Anthopleura elegantissima,* and *F*-statistics for each locus. Within each species, estimates of non-random mating within subpopulations (f) and genetic differentiation among subpopulations (θ) were generally similar across all loci (f and θ could not be calculated for *E. fernaldi* because it was sampled at only two sites). Mean *F*-statistics for the 20 loci (Table 4) revealed extremely large departures from random mating within populations in the remaining three brooding *Epiactis* species (f = 0.831 to 0.957), while departures from random mating in the broadcasting *A. elegantissima* (f = 0.198) were significantly smaller according to the 95% confidence intervals. Mean observed and expected heterozygosities (Table 3) revealed heterozygote deficits in every genetically-variable *Epiactis* population assayed, and in three of the five *A. elegantissima* populations assayed. Average differentiation among populations (Table 4) was highest for the two external brooders (*E. prolifera*, θ = 0.322; *E. lisbethae,* θ = 0.364) and ~50% lower for the internal brooder (*E. ritteri*, θ = 0.157) and the broadcaster (*A. elegantissima*, θ = 0.141). However, only *E. lisbethae* and *A. elegantissima* have non-overlapping 95% confidence intervals for these estimates.

Intra-specific genetic distances (Nei 1978; Table 5) ranged from 0.005 to 0.092 in *Epiactis prolifera*; from

0.068 to 0.093 in *E. lisbethae*; from 0.016 to 0.025 in *E. ritteri;* and from 0.008 to 0.048 in *Anthopleura elegantissima.* In *E. fernaldi*, the two populations surveyed were identical. For each species, a simple regression of genetic distance between pairs of populations on geographic distance between the populations did not detect a correlation. The power of these tests is limited by the small number of populations sampled for most of the species. Further, the regressions are flawed by being based on non-independent pairwise comparisons (see Hellberg 1994). However, even for the species in which the greatest number of populations was sampled (*E. prolifera*), the association between genetic and geographic distance was so weak ($r = 0.018$; $F = 0.011$; $df = 35$; $P = 0.9159$), that it is unlikely that any correlation exists. Similarly, allelic frequencies at individual allozyme loci (Table 3) do not reveal any obvious geographic clines in any species.

DNA-fingerprinting

The average percent difference (APD) between conspecific individuals from the same location was extremely low for the MacGinitie Cave *Epiactis fernaldi* population (0.04) and moderate (0.21 to 0.54) for populations of the remaining four species (Table 6, Fig 2). Where two or more geographic sites were analyzed, between-site variation was generally within the range of within-site variation (cf. Tables 6 and 7).

Allozymes vs DNA-fingerprinting

Summary statistics (Table 8) show that within-location variation for both allozymes (mean expected heterozygosity per population, H_e) and DNA fingerprints (APD_{within}) was lowest in *Epiactis fernaldi* and substantially higher in the remaining four species. DNA fingerprint estimates for between-location differences ($APD_{between}$) are limited to the three species where two or more populations were run on the same gel. For these species, between-location allozyme differentiation (genetic distance, *D*) was greater in *E. prolifera* than in *E. ritteri* or *Anthopleura elegantissima*. DNA-fingerprinting detected the same pattern, but the differences are not significant.

Discussion

Effects of dispersal

We expected all four brooding *Epiactis* species to have less genetic variation within populations and greater differentiation among populations than the broadcasting species *Anthopleura elegantissima.* Results were only partially consistent with these expectations. As predicted, observed allozyme heterozygosities were lower for the four brooders (mean H_o varied from 0.000 to 0.012) than for the broadcaster (mean $H_o = 0.086$). In fact, the observed heterozygosity values in all *Epiactis* species were substantially lower than in most other invertebrates (mean H_o in a survey of 1106 species of invertebrates was 0.100: Nevo et al. 1984), and were especially low for sea anemones, in which high heterozygosities are common across a range of life-history characteristics (mean H_o for 23 species = 0.179, Shick 1991; see also Solé-Cava and Thorpe 1991). However, such extremely low heterozygosity in *Epiactis* spp. is consistent with Bucklin et al.'s (1984) study of *E. prolifera,* in which 11 of 14 enzyme loci were fixed, and the remaining 3 loci had average H_o values of 0.02.

The DNA-fingerprint data show a very different pattern of within-population variation from the allozyme data. While one of the brooding species had strikingly low within-site variation (APD = 0.04 in *Epiactis fernaldi*), the remaining three brooding species had within-site variation (mean APD varied from 0.30 to 0.37) similar to that in the broadcaster *Anthopleura elegantissima* (mean APD = 0.36). Because the DNA-fingerprint data in this study are among the first reported for marine invertebrates, there are few previous examples with which they can be compared, but the level of within-site minisatellite variation in all five anemone species was on the low end of values reported for other taxa, which range from 0.00 in a terrestrial plant (Rogstad et al. 1991) to at least 0.89 in a fish (Baker et al. 1992).

The average level of population subdivision detected by allozymes was lower for *Anthopleura elegantissima* ($\theta = 0.141$) than for either of the externally brooding species (*Epiactis prolifera*: $\theta = 0.322$; *E. lisbethae*: $\theta = 0.364$), although the difference was significant only for *E. lisbethae.* This is consistent with the hypothesis that dispersal and gene flow are restricted in brooding species. More unexpected was the finding that population subdivision of the internal brooder *E. ritteri* ($\theta = 0.157$) was similar to that of the broadcaster. Such high gene flow in a brooder might be caused by occasional long-distance dispersal of newly-released juveniles, particularly if offspring are released at high tide. The possibility that *E. ritteri* may produce dispersive larvae also cannot be ruled out, although this species has never been observed to release larvae. The lack of population differentiation in *E. fernaldi* was also not expected for a brooding species, but may be an artifact of sampling. There was *no* allozyme variation either within or between the two *E. fernaldi* sites sampled, and since they were <10 km apart, they may have been founded from the same ancestral population. Because both *E. fernaldi* sites are log-roofed "caves" (presumably created by storms) on the exposed side of San Juan Island, it has been suggested that these *E. fernaldi* immigrated during rough weather from further north where they may be more abundant (Fautin, personal communication).

For the three species in which more than one population was run on the same DNA fingerprinting gel, between-site minisatellite variation showed the same

Table 3 *Epiactis* spp. and *Anthopleura elegantissima*. Allele frequencies for 20 enzyme loci in each population. Collecting sites, numbered *N* mean sample size; H_o mean observed heterozygosity; H_e mean expected heterozygosity; $^{*}P$ <0.05; $^{**}P$ <0.01; $^{***}P$ <0.001; – zero; *na*

Locus, allele	*E. prolifera* populations									*f*	*θ*	*E. lisbethae* populations		
	2	3	5	8	9	10	11	12	13			5	9	11
CAT										1.000**	0.815**			
A	–	–	1.000	0.050	1.000	0.905	1.000	0.500	1.000			0.867	0.882	1.000
B	1.000	1.000	–	0.950	–	0.095	–	0.500	–			0.133	0.118	–
C	–	–	–	–	–	–	–	–	–			–	–	–
D	–	–	–	–	–	–	–	–	–			–	–	–
EST										1.000**	0.109**			
A	0.176	–	0.200	–	0.259	–	–	–	0.091			–	–	–
B	0.824	1.000	0.800	1.000	0.741	1.000	1.000	1.000	0.909			1.000	1.000	1.000
C	–	–	–	–	–	–	–	–	–			–	–	–
ESTD-1										0.957**	0.259*			
A	0.688	–	0.200	–	0.048	0.190	0.067	–	0.059			–	0.750	–
B	0.312	1.000	0.800	1.000	0.952	0.810	0.933	1.000	0.941			1.000	0.250	1.000
C	–	–	–	–	–	–	–	–	–			–	–	–
D	–	–	–	–	–	–	–	–	–			–	–	–
E	–	–	–	–	–	–	–	–	–			–	–	–
ESTD-2										1.000**	0.068			
A	0.059	–	–	–	0.071	–	0.200	0.100	–			–	0.143	–
B	0.941	1.000	1.000	1.000	0.929	1.000	0.800	0.900	1.000			1.000	0.857	1.000
C	–	–	–	–	–	–	–	–	–			–	–	–
GDH										1.000**	0.013			
A	–	–	–	–	–	–	–	–	–			–	–	–
B	0.933	0.800	0.800	1.000	0.933	1.000	0.933	0.917	1.000			0.533	1.000	1.000
C	0.067	0.200	0.200	–	0.067	–	0.067	0.083	–			0.467	–	–
GOT										0.017	−0.018			
A	1.000	1.000	1.000	1.000	0.983	1.000	1.000	1.000	1.000	–		0.893	1.000	1.000
B	–	–	–	–	–	–	–	–	–	–		–	–	–
C	–	–	–	–	0.017	–	–	–	–			0.107	–	–
G6PDH										0.946**	0.221**			
A	0.964	1.000	1.000	0.905	0.683	0.524	1.000	0.875	1.000			1.000	1.000	1.000
B	0.036	–	–	0.095	0.317	0.476	–	0.125	–			–	–	–
GPI-1										1.000***	0.312**			
A	1.000	1.000	1.000	0.455	0.870	1.000	0.900	1.000	1.000			0.800	1.000	0.200
B	–	–	–	0.545	0.130	–	0.100	–	–			0.200	–	0.600
C	–	–	–	–	–	–	–	–	–			–	–	0.200
D	–	–	–	–	–	–	–	–	–			–	–	–
GPI-2										0.940**	0.293*			
A	1.000	1.000	1.000	0.455	0.870	1.000	0.833	1.000	1.000			0.800	0.824	0.200
B	–	–	–	0.545	0.130	–	0.100	–	–			0.200	–	0.600
C	–	–	–	–	–	–	0.017	–	–			–	0.176	0.200
D	–	–	–	–	–	–	–	–	–			–	–	–
HK										0.971*	0.012			
A	–	–	–	–	–	–	–	–	0.050			0.200	–	–
B	–	–	–	0.036	–	–	–	–	–			–	–	–
C	1.000	1.000	1.000	0.964	1.000	1.000	1.000	1.000	0.950			0.800	1.000	1.000
IDH										0.814**	0.039*			
A	–	–	–	–	–	–	–	–	–			–	–	–
B	–	0.045	–	0.053	0.024	–	–	–	–			0.036	0.033	–
C	1.000	0.955	1.000	0.947	0.976	1.000	0.900	1.000	0.833			0.964	0.967	1.000
D	–	–	–	–	–	–	0.100	–	0.167			–	–	–
MDH-1										1.000*	0.084*			
A	–	–	–	–	–	–	–	–	–			–	–	–
B	1.000	1.000	1.000	0.900	0.900	0.714	0.867	1.000	1.000			1.000	1.000	0.875
C	–	–	–	0.100	0.100	0.286	0.133	–	–			–	–	0.125
MDH-2										1.000*	0.096*			
A	–	–	0.250	–	–	–	–	–	–			–	–	–
B	1.000	1.000	0.750	0.905	1.000	0.714	0.867	0.909	1.000			1.000	1.000	1.000
C	–	–	–	0.095	–	0.286	0.133	0.091	–			–	–	–

(*E. prolifera* and *E. lisbethae* data are continued on p. 492)

from north to south, are listed in Table 2 [f (=F_{IS}) inbreeding within subpopulations; θ (= F_{ST}) inbreeding due to population subdivision; not applicable. No F-statistics are shown for *E. fernaldi* because only two populations were sampled]

f	θ	*E. ritteri* populations			f	θ	*E. fernaldi* populations		*A. elegantissima* populations					f	θ
		1	8	10			4	7	5	6	9	10	14		
1.000**	−0.024				na	na								0.118	0.076**
		–	–	–			–	–	–	–	–	–	–		
		–	–	–			–	–	–	0.121	–	0.143	–		
		–	–	–			–	–	1.000	0.879	1.000	0.857	1.000		
		1.000	1.000	1.000			1.000	1.000	–	–	–	–	–		
na	na				na	na								−0.078	0.064
		–	–	–			1.000	1.000	–	–	–	–	–		
		1.000	1.000	1.000			–	–	–	–	0.069	–	–		
		–	–	–			–	–	1.000	1.000	0.931	1.000	1.000		
0.826**	0.691***				na	na								0.410***	0.131***
		–	–	–			–	–	–	–	–	–	–		
		–	–	–			–	–	–	–	–	–	–		
		–	–	–			–	–	0.967	1.000	0.775	0.750	1.000		
		1.000	1.000	1.000			1.000	1.000	–	–	–	–	–		
		–	–	–			–	–	0.033	–	0.225	0.250	–		
1.000***	0.060				1.000***	0.023								0.242	0.157***
		–	–	0.120			–	–	–	–	–	–	–		
		1.000	1.000	0.760			–	–	0.020	–	–	0.192	–		
		–	–	0.120			1.000	1.000	0.980	1.000	1.000	0.808	1.000		
1.000***	0.393***				0.360*	0.057*								0.240	0.019
		1.000	0.812	0.964			1.000	1.000	–	0.022	0.052	0.059	–		
		–	0.188	0.036			–	–	1.000	0.978	0.948	0.941	1.000		
		–	–	–			–	–	–	–	–	–	–		
0.651*	0.040				0.017	−0.028								0.582***	0.098***
		–	–	–			1.000	1.000	–	–	–	–	–		
		1.000	1.000	0.975			–	–	1.000	1.000	0.860	0.763	0.950		
		–	–	0.025			–	–	–	–	0.140	0.237	0.050		
na	na				na	na								0.123	0.066
		1.000	1.000	1.000			1.000	1.000	0.875	1.000	0.948	1.000	1.000		
		–	–	–			–	–	0.125	–	0.052	–	–		
1.000***	0.458***				1.000***	0.284***								na	na
		–	–	–			–	–	1.000	1.000	1.000	1.000	1.000		
		0.286	–	–			–	–	–	–	–	–	–		
		–	–	–			–	–	–	–	–	–	–		
		0.714	1.000	1.000			1.000	1.000	–	–	–	–	–		
1.000***	0.255***				1.000***	0.284***								−0.021	0.439***
		–	–	–			–	–	1.000	1.000	0.346	1.000	1.000		
		0.286	–	–			–	–	–	–	0.462	–	–		
		–	–	–			–	–	–	–	0.192	–	–		
		0.714	1.000	1.000			1.000	1.000	–	–	–	–	–		
1.000***	0.107*				na	na								0.447**	0.102*
		–	–	–			1.000	1.000	–	–	–	–	–		
		1.000	1.000	1.000			–	–	–	–	–	0.111	–		
		–	–	–			–	–	1.000	1.000	1.000	0.889	1.000		
0.004	−0.026				0.798***	0.175***								−0.148	0.095**
		–	–	–			–	–	–	–	0.096	–	–		
		0.429	0.063	0.045			1.000	1.000	–	–	–	–	–		
		0.571	0.937	0.932			–	–	1.000	1.000	0.885	1.000	0.833		
		–	–	0.023			–	–	–	–	0.019	–	0.167		
1.000*	0.077*				1.000***	−0.033								0.087	0.190***
		–	–	–			–	–	0.370	0.093	0.548	0.344	–		
		0.846	1.000	0.857			1.000	1.000	0.608	0.907	0.452	0.656	1.000		
		0.154	–	0.143			–	–	0.022	–	–	–	–		
na	na				1.000*	−0.023								−0.343**	0.201***
		–	–	–			–	–	–	–	0.019	–	–		
		0.923	1.000	1.000			–	–	1.000	0.925	0.962	0.933	0.611		
		0.077	–	–			1.000	1.000	–	0.075	0.019	0.067	0.389		

(*E. ritteri*, *E. fernaldi* and *A. elegantissima* data are continued on p. 493)

Table 3 (continued)

Locus, allele	*E. prolifera* populations									*f*	*θ*	*E. lisbethae* populations		
	2	3	5	8	9	10	11	12	13			5	9	11
ME										na	na			
A	–	–	–	–	–	–	–	–	–			–	–	–
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000			1.000	1.000	1.000
C	–	–	–	–	–	–	–	–	–			–	–	–
PEP-1										1.000**	0.241**			
A	–	–	–	–	–	–	–	–	–			–	–	–
B	–	–	–	–	–	–	–	–	–			–	–	–
C	–	0.167	–	–	–	–	–	–	–			0.867	0.125	0.400
D	1.000	0.833	1.000	1.000	1.000	1.000	1.000	1.000	1.000			0.133	0.875	0.600
PEP-2										na	na			
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000			0.867	0.875	0.900
B	–	–	–	–	–	–	–	–	–			0.133	0.125	0.100
C	–	–	–	–	–	–	–	–	–			–	–	–
PGDH										1.000**	0.024*			
A	0.941	1.000	1.000	1.000	1.000	1.000	1.000	0.917	1.000			1.000	1.000	0.600
B	–	–	–	–	–	–	–	0.083	–			–	–	0.400
C	0.059	–	–	–	–	–	–	–	–			–	–	–
PGM										0.821**	–0.010			
A	1.000	1.000	1.000	1.000	0.952	1.000	0.933	0.942	1.000			1.000	1.000	1.000
B	–	–	–	–	0.048	–	0.067	0.029	–			–	–	–
C	–	–	–	–	–	–	–	0.029	–			–	–	–
SOD										1.000**	0.015*			
A	–	–	–	–	–	–	–	–	–			–	–	–
B	1.000	1.000	1.000	1.000	1.000	1.000	0.933	1.000	1.000			1.000	1.000	0.900
C	–	–	–	–	–	–	0.067	–	–			–	–	0.100
XDH										na	na			
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000			1.000	1.000	1.000
B	–	–	–	–	–	–	–	–	–			–	–	–
(N)	(16.0)	(11.2)	(9.9)	(15.3)	(27.4)	(20.6)	(30.0)	(21.0)	(21.5)			(13.3)	(15.2)	(7.8)
H_o	0.010	0.005	0.000	0.009	0.006	0.000	0.002	0.006	0.000			0.007	0.007	0.000
H_e	0.059	0.036	0.071	0.092	0.096	0.097	0.094	0.076	0.033			0.125	0.084	0.140

pattern as between-site allozyme variation: population differences in *Anthopleura elegantissima* were similar to those in *Epiactis ritteri* and lower than those in *E. prolifera.* However, these interspecific differences were not significant and the levels of within and between site variation overlapped in this study, as has been previously reported for fish populations (Baker et al. 1992). In cases such as these, DNA-fingerprinting may not be particularly effective for detecting population subdivision.

Effects of mating system

As with dispersal, the results were only partially consistent with expectations for different mating systems: that selfing organisms should have greater population subdivision and greater local inbreeding than outcrossing organisms. While both the allozyme and DNA data showed a trend toward greater population subdivision in the self-fertile *Epiactis prolifera* than in the outcrosser *E. ritteri*, the outcrosser *E. lisbethae* had the

Table 4 *Epiactis* spp. and *Anthopleura elegantissima*. Mean *F*-statistics estimates for 20 allozyme loci [$f = F_{IS}$ (inbreeding within subpopulations); $\theta = F_{ST}$ (inbreeding due to population subdivision); $F = F_{IT}$ (total inbreeding); *CI* confidence interval]

	E. prolifera	*E. lisbethae*	*E. ritteri*	*A. elegantissima*
$f \pm$ SE	0.955 ± 0.018	0.957 ± 0.008	0.831 ± 0.092	0.198 ± 0.069
(95% CI)	(0.910 – 0.983)	(0.879 – 1.000)	(0.622 – 0.968)	(0.063 – 0.328)
$\theta \pm$ SE	0.322 ± 0.156	0.364 ± 0.021	0.157 ± 0.042	0.141 ± 0.023
(95% CI)	(0.098 – 0.507)	(0.181 – 0.476)	(0.078 ± 0.233)	(0.094 – 0.180)
$F \pm$ SE	0.970 ± 0.016	0.972 ± 0.005	0.858 ± 0.079	0.311 ± 0.067
(95% CI)	(0.929 ± 0.989)	(0.921 – 1.000)	(0.671 – 0.975)	(0.181 – 0.444)

f	θ	*E. ritteri* populations			f	θ	*E. fernaldi* populations		*A. elegantissima* populations					f	θ
		1	8	10			4	7	5	6	9	10	14		
na	na				na	na								0.442**	0.053*
		1.000	1.000	1.000			1.000	1.000	–	–	–	–	–		
		–	–	–			–	–	0.917	1.000	1.000	0.861	1.000		
		–	–	–			–	–	0.083	–	–	0.139	–		
1.000***	0.491***				1.000*	0.436**								0.444***	0.041*
		–	–	–			1.000	1.000	–	–	–	–	–		
		–	0.333	–			–	–	0.036	0.077	0.306	0.158	0.115		
		1.000	0.667	1.000			–	–	0.036	0.115	–	–	–		
		–	–	–			–	–	0.928	0.808	0.694	0.842	0.885		
1.000**	−0.078				0.142	0.022								0.539**	0.047*
		1.000	0.875	0.875			1.000	1.000	0.857	0.860	1.000	0.853	0.833		
		–	0.125	0.125			–	–	0.143	–	–	0.118	–		
		–	–	–			–	–	–	0.140	–	0.029	0.167		
1.000**	0.383***				na	na								0.189	0.068**
		–	–	–			–	–	0.980	0.944	1.000	0.853	1.000		
		1.000	1.000	1.000			1.000	1.000	–	–	–	–	–		
		–	–	–			–	–	0.020	0.056	–	0.147	–		
na	na				1.000*	0.269**								−0.246	0.133***
		1.000	1.000	0.625			1.000	1.000	0.977	0.714	0.952	0.938	1.000		
		–	–	–			–	–	0.023	0.238	–	0.062	–		
		–	–	0.375			–	–	–	0.048	0.048	–	–		
1.000*	0.020				1.000*	0.277**								0.672**	0.064***
		1.000	0.750	1.000			1.000	1.000	–	–	–	–	–		
		–	0.250	–			–	–	1.000	1.000	1.000	0.921	1.000		
		–	–	–			–	–	–	–	–	0.079	–		
na	na				na	na								na	na
		–	–	–			–	–	–	–	–	–	–		
		1.000	1.000	1.000			1.000	1.000	1.000	1.000	1.000	1.000	1.000		
		(11.5)	(6.7)	(19.9)			(18.8)	(9.4)	(19.3)	(19.7)	(25.1)	(16.5)	(10.5)		
		0.007	0.013	0.023			0.000	0.000	0.077	0.073	0.095	0.115	0.072		
		0.089	0.082	0.082			0.000	0.000	0.074	0.085	0.146	0.178	0.070		

highest level of population subdivision for allozymes (no interpopulation DNA comparisons were done for this species). Levels of local inbreeding were also not consistent with expectations based on mating systems. High levels of local inbreeding were detected in all three genetically variable *Epiactis* species (f = 0.831 to 0.957), reflecting the heterozygote deficits detected at virtually every locus. Evidence of local inbreeding was expected for *E. prolifera*, since it is capable of self-fertilization and substantial heterozygote deficits were known from a previous study (average of 28.1%: Bucklin et al. 1984). The extreme departures from random mating were not expected for the outcrossing *E. lisbethae* and *E. ritteri.* Heterozygote deficiencies are common in a variety of apparently outcrossing marine taxa, and have been attributed to null alleles (Gardner 1992), selection against heterozygotes (Zouros and Foltz 1984), scoring bias (Buroker et al. 1975), geographic subdivision/mixing of genetically differentiated groups (the Wahlund effect: Koehn et al. 1976), synchronized spawning based on genotype (Zouros and Foltz 1984) or highly localized fertilization of eggs by near neighbors before larval dispersal (Smith and Potts 1987). However, reported examples of these explanations have substantially lower heterozygote deficits than those found in *E. lisbethae* and *E. ritteri*. Instead, the genetic pattern in these *Epiactis* species more closely resembles that found in terrestrial plants with limited pollen flow, where severe heterozygote deficits in self-incompatible species have been attributed to cross-fertilization between neighbors in small, highly subdivided populations (Schaal and Levin 1976; Levin 1978).

The moderate heterozygote deficits found in *Anthopleura elegantissima* (even after duplicate 20-locus genotypes had been removed) are similar to those found in many other outcrossing marine taxa (e.g. Skibinski et al. 1983; Garton 1984). Other studies of *A. elegantissima* have attributed heterozygote deficits to highly localized fertilization of eggs before larval dispersal (Smith and Potts 1987) or to null alleles (Grosberg, McFadden and Cameron personal communication). A third potential cause may be a Wahlund effect due to temporal or geographic variation in allele frequencies, as has been suggested for other freespawning species with long-lived, pelagic larvae (Johnson and Black 1984; Ayre and Dufty 1994).

Table 5 *Epiactis* spp. and *Anthopleura elegantissima.* Unbiased genetic distances (*above diagonal*) and identities (*below diagonal*) (Nei 1978) between all pairs of conspecific populations in each of five species. Data are for 20 allozyme loci. Populations numbered from north to south (*I* Island; *Pt* Point)

Epiactis prolifera	Population:								
	2	3	5	8	9	10	11	12	13
2. Neck Pt		.027	.070	.060	.085	.080	.083	.040	.077
3. Brown I	.973		.061	.035	.068	.070	.062	.016	.058
5. Mar Vista	.932	.941		.092	.011	.023	.009	.020	.006
8. Tatoosh I	.942	.966	.912		.077	.088	.076	.044	.084
9. Cape Arago	.919	.935	.989	.926		.014	.010	.021	.009
10. Bodega Bay	.923	.932	.978	.916	.987		.019	.024	.022
11. Pigeon Pt	.920	.940	.991	.927	.990	.981		.017	.005
12. Ship Rock	.961	.984	.980	.957	.979	.977	.983		.017
13. Bird Rock	.926	.944	.994	.919	.991	.978	.995	.984	

Epiactis lisbethae	Population:		
	5	9	11
5. Mar Vista		.080	.068
9. Cape Arago	.924		.093
11. Pigeon Pt	.934	.911	

Epiactis ritteri	Population:		
	1	8	10
1. Execution Rocks		.024	.025
8. Tatoosh I	.976		.016
10. Bodega Bay	.976	.984	

Epiactis fernaldi	Population:	
	4	7
4. MacGinitie Cave		.000
7. South Beach	1.000	

Anthopleura elegantissima	Population:				
	5	6	9	10	14
5. Mar Vista		.010	.027	.008	.018
6. Eagle Pt	.990		.041	.014	.010
9. Cape Arago	.974	.960		.029	.048
10. Bodega Bay	.993	.986	.971		.022
14. Fishermen's Cove	.982	.990	.953	.979	

The extent to which a geographic Wahlund effect explains the heterozygote deficiencies in all five species can be estimated by the ratio of the variance in allele frequencies among individual collections to the observed heterozygote deficits [i.e., $\Sigma\sigma^2/(H_e\text{-}H_o)$, where H_e is the heterozygosity expected at Hardy–Weinberg equilibrium and H_o is the mean heteozygosity observed within locations (see Li 1976; Johnson and Black 1984; Ayre and Dufty 1994)]. This accounts for up to half of the heteorzygote deficits in each species (24.2% in *Epiactis prolifera*; 42.8% in *E. lisbethae*; 42.0% in *E. ritteri*; 50.7% in *Anthopleura elegantissima)*. However, these would be underestimates if individual collections themselves contained multiple subpopulations. This could best be tested by a hierarchical, spatial sampling procedure, yet the rarity of the *Epiactis* species makes this inadvisable. The possibility that the remarkably large heterozygote deficits in the *Epiactis* species were due to biased scoring of allozymes is unlikely, since *A. elegantissima* had a much smaller deficit. Other possible causes of the significant *f* values (e.g. selection against heterozygotes, synchronized spawning of genotypes, null alleles) have yet to be investigated, but the consistency of heterozygote deficits across loci argues against such explanations.

Table 6 *Epiactis* spp. and *Anthopleura elegantissima.* DNA-fingerprint variation within populations. Only samples run on same gel are compared. *E. prolifera* population at Pigeon Point was probed with M13; all others were probed with Jeffreys' 33.6 probe (*N* number of individuals; *f* average number of scoreable bands per individual; *APD* average percent difference for all pairwise comparisons of individual fingerprints; *SE* standard error calculated according to Lynch 1990)

Species, Population	(*N*)	*f*	APD(SE)
E.prolifera			
2. Neck Pt	(4)	24.3	0.21(0.06)
3. Brown I	(4)	20.3	0.28(0.20)
5. Mar Vista	(4)	19.5	0.43(0.21)
9. Cape Arago	(5)	10.8	0.21(0.10)
11. Pigeon Pt	(13)	15.1	0.54(0.13)
E. lisbethae			
9. Cape Arago	(8)	12.3	0.30(0.13)
E. ritteri			
8. Tatoosh I	(5)	16.4	0.41(0.09)
10. Bodega Bay	(6)	14.5	0.32(0.13)
E. fernaldi			
4. MacGinitie Cave	(6)	14.0	0.04(0.02)
A. elegantissima			
9. Cape Arago	(6)	9.8	0.39(0.15)
10. Bodega Bay	(7)	9.0	0.33(0.19)

Table 7 *Epiactis* spp. and *Anthopleura elegantissima.* DNA-fingerprint variation between conspecific populations. See Table 6 legend for details

Species	Population 1 (*N*)	Population 2 (*N*)	APD(SE)
E. prolifera	3. Brown I (4)	5. Mar Vista (4)	0.58(0.03)
	2. Neck Pt (4)	3. Brown I (4)	0.22(0.20)
	2. Neck Pt (4)	5. Mar Vista (4)	0.60(0.08)
E. ritteri	8. Tatoosh I (5)	10. Bodega Bay (6)	0.38(0.11)
A. elegantissima	9. Cape Arago (8)	10. Bodega Bay (6)	0.39(0.17)

Interaction between dispersal and mating systems

Compared with marine invertebrates that have limited larval dispersal (e.g. Grosberg 1991; Hellberg 1995) or no larval dispersal (e.g. Hunt 1993; Ayre and Dufty 1994), these *Epiactis* species appear to have generally comparable levels of population subdivision, but sub-

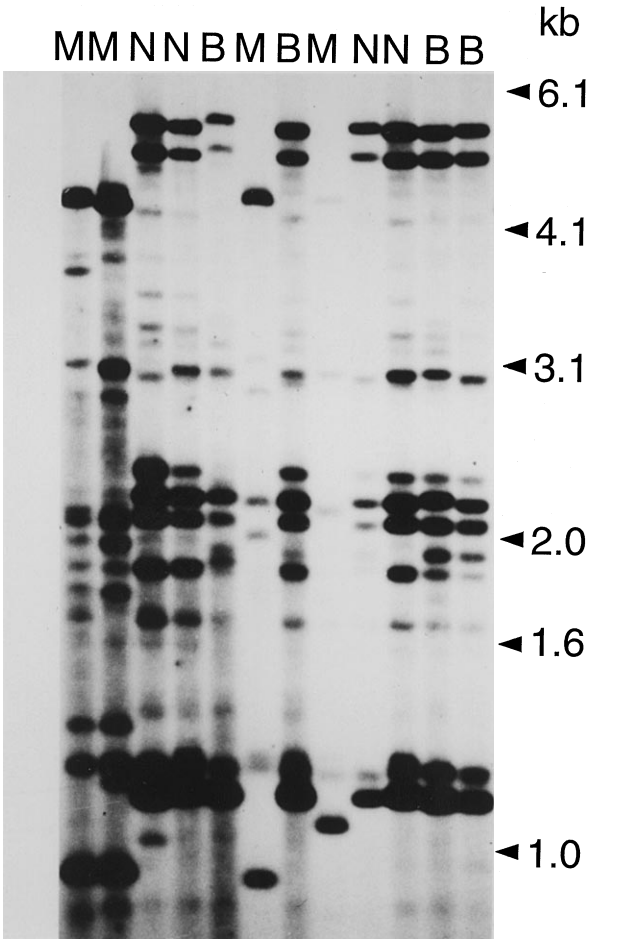

Fig. 2 *Epiactis prolifera.* DNA fingerprints of individuals from three sites in San Juan Islands, Washington (*B* Brown Island; *M* Mar Vista; *N* Neck Point)

Table 8 *Epiactis* spp.and *Anthopleura elegantissima.* Summary of genetic variation (± SE) within and between geographic locations as assessed by allozymes d DNA-fingerprinting. The number of locations sampled are in parentheses. Fingerprint results are for individuals run on same gel, and are limited to gels probed with Jeffreys' 33.6 [H_e mean expected heterozygosity; *D* mean genetic distance (Nei 1978); *APD* average percent difference for all pairwise comparisons of individual fingerprints]

Species	Allozymes		DNA fingerprinting	
	H_e	*D*	APD_{within}	$APD_{between}$
E. prolifera	0.07 ± 0.01(9)	0.05 ± 0.01(9)	0.33 ± 0.07(5)	0.47 ± 0.12(3)
E. lisbethae	0.12 ± 0.02(3)	0.08 ± 0.01(3)	0.30 ± 0.13(1)	–
E. ritteri	0.08 ± 0.00(3)	0.02 ± 0.00(3)	0.37 ± 0.05(2)	0.38 ± 0.11(2)
E. fernaldi	0.00 ± 0.00(2)	0.00 ± 0.00(2)	0.04 ± 0.02(1)	–
A. elegantissima	0.11 ± 0.02(5)	0.02 ± 0.00(5)	0.36 ± 0.03(2)	0.39 ± 0.17(2)

stantially higher levels of local inbreeding. This pattern might be caused by occasional long-distance dispersal followed by highly localized recruitment within established populations. One mechanism of long-distance dispersal may be the rafting of adults. Rafting may be facilitated by the occurrence of *Epiactis* spp. on eelgrass or algae (Dunn 1977b; Fautin and Chia 1986), or on stranded logs, all of which may tear loose and float, especially after storms. Such events have the potential to cause population bottlenecks and small effective population sizes. The complete lack of a larval dispersal stage may then promote subsequent population subdivision on a very fine spatial scale, particularly in the external brooders whose offspring crawl directly onto the surrounding substrate, where they may form tight groupings of interbreeding relatives. Such a population structure results in "effective selfing" (sensu Ritland 1984), even in obligate outcrossers, and may promote the evolution of true selfing (Shields 1982; Strathmann et al. 1984; Uyenoyama 1986). DNA analyses which detected little or no variation within broods (Edmands 1995) suggest that *Epiactis* species are highly inbred (or possibly asexual), making it difficult to define an adaptive advantage for the maintenance of separate sexes and cross-fertilization in this system.

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