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# Population genetics and systematics of the *Leptasterias hexactis* (Echinodermata: Asteroidea) species complex *

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**Abstract.** Morphological characters and 14 enzyme loci were examined for 1 040 sea stars, currently recognized as forms of *Leptasterias hexactis*, from Lynn Canal, Alaska, and Puget Sound, Washington, USA, between March 1988 and April 1989. Three morphologically and two genetically distinct *Leptasterias* forms were identified. The most common form found at both localities was *L. epichlora* (Brandt) *sensu* Verrill. *L. hexactis* (Stimpson) *sensu* Verrill co-occurred with *L. epichlora* at all study sites and apparently hybridizes extensively with *L. epichlora* in the Puget Sound region, but rarely, if at all, in Alaska. The presumptive product of this hybridization morphologically resembled *L. aequalis* (Stimpson) *sensu* Fisher, and was conspicuously absent from Alaskan samples. Considerable genetic distance existed between *L. epichlora* and *L. hexactis* (Nei's $D=0.19\pm0.01$) and moderate genetic differentiation occurred between populations of each species from Alaska and Washington (Weir and Cockerham's $F_{RT}=0.29\pm0.04$ for *L. epichlora* and $0.21\pm0.15$ for *L. hexactis*). A significant ($p<0.05$) deficiency in the proportion of heterozygous individuals was found compared to Hardy-Weinberg expectations (Wright's fixation index, $F_{ID}=0.12\pm0.04$ and $0.31\pm0.08$ for *L. epichlora* and *L. hexactis*, respectively). However, mean observed heterozygosity for each species ($0.09\pm0.03$, $0.14\pm0.04$ and $0.14\pm0.04$ for *L. epichlora*, *L. hexactis* and *L. aequalis*, respectively) fell within the range of reported values for other asteroid species (ca. 0.04 to 0.37). The results of this study indicate that considerable genetic integrity is maintained between *L. epichlora* and *L. hexactis*, which warrants their recognition as distinct species despite their apparent hybridization in the Puget Sound region.

## Introduction

The systematics of small six-rayed sea stars from the North Pacific Ocean, genus *Leptasterias*, have been controversial for many years, particularly in the Puget Sound region where species identification based on morphological characters is compromised because of presumed hybridization (Verrill 1914, Fisher 1930, Chia 1966). Prior to 1966, three species, *L. epichlora* (Brandt, 1835), *L. hexactis* (Stimpson, 1862a), and *L. aequalis* (Stimpson, 1862b) were recognized in the Puget Sound region (Verrill 1914, Bush 1918, 1921, Fisher 1930). Chia (1966) synonymized these species based on the ability of female *L. hexactis* and male *L. aequalis* to interbreed in the laboratory, with hybrid progeny showing no developmental abnormalities through metamorphosis, and on their sharing a common ecological niche and concomitant breeding seasons; the name *L. hexactis* has taken precedence.

All the aforementioned systematic investigators, except Chia (1966), used only morphological characters in the identification of species. These characters are known to be extremely variable, especially in the Puget Sound region (Verrill 1914, Fisher 1930, Chia 1966). We investigated the systematic status of the *Leptasterias* species using both morphological and electrophoretic (allozyme) data. Allozymic characters have been used to identify cryptic sibling species in a number of invertebrate taxa (Manwell and Baker 1963, Steiner et al. 1977, Chambers 1978, Thorpe et al. 1978, McDonald and Koehn 1988), including sea stars (Schopf and Murphy 1973, Tuttle and Lindahl 1980).

The purpose of this study was to determine (1) the number of morphologically distinct forms of *Leptasterias* spp. in samples from Alaska and Washington, (2) the amount of genetic differentiation both within and among these forms, and (3) the proper taxonomic rank and name for each form. Electrophoretic techniques were employed to confirm the systematic status of each of the morphological types. To avoid ambiguity, we refer to these forms by their older taxonomic names, *L. epichlora*, *L. hexactis*, and *L. aequalis*, without the recognition of subspecies, formae or varieties.

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## Materials and methods

*Leptasterias* spp. were obtained from Lynn Canal, Alaska, and San Juan Island, Washington, separated by ca. 1500 km (see Kwast 1989 for exact locations). At each collection site, all *Leptasterias* spp. encountered along the rocky intertidal were collected, until more than 100 individuals were obtained. Individuals were collected from five sites (Bridget Cove, Sunset Cove, Benjamin Island, Eagle River and Auke Bay), each separated by less than 25 km, along Lynn Canal in southeastern Alaska, between March and September 1988. Samples were transferred to the laboratory in insulated containers at 5° to 8 °C, and maintained in separate aquaria containing artificial sea water (Instant Ocean; Eastlake, Ohio, USA) of 30‰ S at 13 °C. *Mytilus trossulus* Gould were provided as food until morphological observations were made. More than 100 individuals were also collected from each of four sites (Lonesome Cove, Friday Harbor, False Bay and Mitchell Bay), separated by less than 15 km, on San Juan Island, Washington, in June 1988; these collection sites coincide with those of Chia (1966). Samples were maintained in separate tanks with running sea water until morphological observations were made.

Individuals were identified to species under a dissecting microscope, based on ray shape, coloration, arrangement and abundance of minor and major pedicellariae, spination and body size (Verrill 1914, Bush 1918, 1921, Fisher 1930, Chia 1966, Lambert 1981). Wet weight (g), $R$ (the distance in mm from the center of the disc to the tip of the longest ray) and $r$ (the distance in mm from the center of the disc to the edge of the interradii) were measured, and the $R$ ratio ($R:r$) was calculated. Immediately following morphological examination, individuals were snap-frozen on dry ice and stored at −70 °C until preparation for electrophoresis.

Procedures for tissue-extract preparation, horizontal starch-gel (12% Sigma starch) electrophoresis, and histochemical staining were similar to those of Selander et al. (1971) and Harris and Hopkinson (1976), with minor modifications (Kwast 1989). An initial screening of more than 30 enzymes yielded 14 enzyme loci that produced consistently interpretable results. Isozymes coded by separate loci were numbered in order of decreasing mobility, and were presumed to be homologous among species after exhibiting identical mobilities using several different buffer systems. Electromorphs were equated with alleles and expressed in terms of mobility relative to the most common allele (*100*).

Allele frequencies and observed heterozygosity ($H_o$) per locus per individual were determined by direct count. Mean observed heterozygosity ($\bar{H}_o$) for each species was estimated using average values for all samples, and expected heterozygosity ($H_e$) was calculated from allele frequencies with correction for small sample size (Levene 1949). Weir and Cockerham's (1984) hierarchical $F$-statistics were calculated, with minor modifications (Table 1), using three levels of population structure: individuals within demes ($F_{ID}$, a measure of departure of genotypic frequencies from Hardy-Weinberg expectations); demes within regions (states) ($F_{DR}$); and regions within the total area sampled ($F_{RT}$). Weighted mean $F$-statistics across loci and sampling standard errors were also calculated for each species as per Weir and Cockerham. Unbiased genetic identity ($I$) and distance ($D$) were calculated for pairwise combinations of samples according to Nei (1978). A phenogram based on genetic identity was constructed using the unweighted pair-group method with arithmetic means (UPGMA) (Sneath and Sokal 1973).

Data were reduced and displayed using canonical discriminant analysis. This technique allows for the graphical representation of individuals thought to be hybrids, in this case *Leptasterias aequalis*, between two or more parental species and for the calculation of confidence intervals about mean canonical values for each species (Smouse 1972). Each allele was treated as a separate variable and assigned a number (*0*, *1* or *2*) corresponding to the number of copies of that allele carried by an individual. A canonical discriminant function (PROC CANDISC; SAS Institute, Inc. 1985) was obtained using the parental species *L. epichlora* and *L. hexactis* from both the Alaskan and Washington populations, and the variables $R$ ratio and individual alleles for each polymorphic locus. Similar results were obtained using only Alaskan parental species, but are not reported here. The resultant raw canonical coefficient matrix was then multiplied by the data matrix to obtain individual scores, and these scores were plotted for each state and all locations combined. The canonical function was structured such that individual scores had within-class unit variance. Ninety-five percent confidence intervals were calculated as $\bar{x} \pm Z_{(\alpha=0.05)} \cdot \sigma$, where $\bar{x}$ is the mean canonical score for each respective species, $Z_{(\alpha=0.05)}$ is the ninety-fifth percentile of the standard normal distribution, and $\sigma$ is the standard deviation. In addition, a stepwise canonical discriminant analysis (SAS Institute, Inc. 1985) was performed to determine which variables were most important in discriminating between species.

## Results

### Morphological observations

Two groups, *Leptasterias epichlora* and *L. hexactis*, were distinguished morphologically in Alaska, and an additional group, *L. aequalis*, was distinguished in Washington. General characters shared by all species were six rays, either monocanthid or diplocanthid adambulacral spines (1 or 2 spines per plate, respectively), and minor pedicellariae on the adambulacral spines. A summary of morphological and allozymic differences among the species is presented in Table 2. The most obvious distinguishing characters among the species were the shape of the rays and body size. *L. epichlora* had short stout rays and was, in general, smaller in size than *L. hexactis* for both the Alaskan and Washington populations. Both *L. hexactis* and *L. aequalis* had, in general, longer and more slender rays than *L. epichlora*, although ray shape was highly variable in *L. aequalis*.

Coloration of the aboral surface was variable in all three forms, especially *Leptasterias aequalis* (Table 2). One of the more prominent features which distinguished *L. aequalis* from the other two forms was the presence of multiple chevron stripes on the rays, pointing in the direction of the ray tip. The color of the chevrons was variable, including orange, light blue, indigo and black, but generally darker than the rest of the individual. Whereas *L.*

**Table 1.** $F$-statistics; adapted from Weir and Cockerham (1984). $F_{RT}$: genetic differentiation among regions (states) within total area sampled; $F_{DR}$: differentiation among demes within regions; $F_{ID}$: differentiation among individuals within a deme

| Source of variation | Variance component | $F$-statistic |
|---|---|---|
| Regions ($R$) within total ($T$) | $\sigma^2_{RT}$ | $F_{RT} = \sigma^2_{RT}/\sigma^2_{GT}$ |
| Demes ($D$) within regions | $\sigma^2_{DR}$ | $F_{DR} = \sigma^2_{DR}/(\sigma^2_{GT} - \sigma^2_{RT})$ |
| Individuals ($I$) within demes | $\sigma^2_{ID}$ | $F_{ID} = \sigma^2_{ID}/(\sigma^2_{GT} - \sigma^2_{RT} - \sigma^2_{DR})$ |
| Genes ($G$) within individuals | $\sigma^2_{GI}$ | |
| Total | $\sigma^2_{GT}$ | |

**Table 2.** *Leptasterias* spp. Summary of morphological and allozymic differences among *Leptasterias* species from Alaska and Washington. Underlined alleles indicate fixed differences between *L. epichlora* and *L. hexactis*. *R*: distance in mm from center of disc to tip of longest ray; *r*: distance in mm from center of disc to edge of interradii; *R* ratio: *R*:*r*

| | *L. epichlora* | *L. hexactis* | *L. aequalis* |
|---|---|---|---|
| Distribution | most common species in both Alaskan and Washington samples | least common species in both Alaskan and Washington samples | absent from Alaskan samples; intermediate abundance in Washington samples |
| Ray shape | short, stout | long, slender, often tapered | long, slender, somewhat resembling *L. hexactis;* variable |
| Coloration and markings | mottled indigo, blue-gray or dark green (variable) | uniform dark olive-green or indigo | olive-green, coral red, orange, indigo or gray; distinct chevron stripes (highly variable) |
| Podia coloration | pale yellow or white | pale yellow or white; Alaskan specimens blue | pale yellow, white or light brown |
| Minor pedicellariae | few; random arrangement around aboral spines; form clusters around superomarginal and inferomarginal spines | most numerous; often embedded in tissue; form characteristic wreath-like arrangement around aboral spines | numerous (variable); random arrangement around aboral spines |
| Major pedicellariae | few on aboral surface; few on adambulacral spines | more numerous on aboral surface; abundant around adambulacral spines | few on aboral surface; some on adambulacral spines |
| Spination[a] | | | |
| abactinal | fewer; striated | fewer; striated | numerous; striated |
| carinal | do not form series | form long regular series | do not form series |
| superomarginal | form series; 1 to each plate | form series; 1 to each plate | form series; 1 or 2 to each plate |
| Allozymes | *Pep-1* 90, 95, 100<br>*Pgi* 100[b]<br>*Ipo-2* 78, 100, 144 | *Pep-1* 103, 107, 112<br>*Pgi* 60[b]<br>*Ipo-2* 100, 122 | *Pep-1* 95, 100, 103, 107, 112<br>*Pgi* 60, 100<br>*Ipo-2* 100, 122, 144 |
| Morphometrics | | | |
| Wet wt[c] $\bar{x}$ = | 3.66 | 8.66 | 3.29 |
| range = | (0.30–18.60) | (0.70–33.81) | (0.50–11.30) |
| $R$[d] $\bar{x}$ = | 2.26 | 3.23 | 2.28 |
| range = | (0.90–4.70) | (1.0–6.0) | (1.0–4.5) |
| $r$[d] $\bar{x}$ = | 0.67 | 0.88 | 0.64 |
| range = | (0.30–1.50) | (0.40–1.80) | (0.30–1.20) |
| $R$ ratio $\bar{x}$ = | 3.43 | 3.69 | 3.59 |
| range = | (1.71–5.71) | (1.71–7.00) | (2.00–6.20) |

[a] Adapted from Chia (1964)
[b] Exceptions for certain populations (Lonesome Cove and Friday Harbor) noted in "Results – Electrophoretic analysis: Allele frequencies"
[c] Measured in grams
[d] Measured in millimeters

*epichlora* often had a mottled appearance, the chevrons of *L. aequalis* were distinct and diagnostic for this form. The coloration of the podia separated the two forms in Alaska, in that nearly all (94%) *L. hexactis* examined from Alaska had a blue tint to the podia whereas *L. epichlora* had pale yellow or white podia.

The most consistent morphological difference between the three groups was the arrangement and abundance of crossed minor pedicellariae around the aboral spines. The minor pedicellariae of *Leptasterias hexactis* were generally embedded in tissue either at the base or approximately midway on the spine, and formed a characteristic wreath-like arrangement around the spines. The minor pedicellariae of *L. epichlora* were fewer in number, randomly arranged over the entire aboral surface, and often free-standing (not embedded in tissue) at or near the base of the spines, while those of *L. aequalis* were randomly arranged and more numerous than in *L. epichlora*. *L. epichlora* and *L. hexactis* lacked the two to three enlarged lateral teeth on the terminal lip of the minor pedicellariae which Fisher (1930) reports as being characteristic of *L. aequalis*. Many *L. aequalis* examined exhibited this trait, but this feature was not diagnostic since it was lacking in numerous individuals that resembled *L. aequalis* for all other traits examined.

The major pedicellariae are straight (not crossed), larger in size, and fewer in number than the minor pedicellariae. They are most abundant on the adambulacral and oral spines although they may occur on the aboral surface. Many individuals of all three forms lacked any major pedicellariae. However, the major pedicellariae occurred more frequently and were more abundant for *Leptasterias hexactis* than for either *L. epichlora* or *L. aequalis*.

**Table 3.** *Leptasterias* spp. Summary of morphometric data for Alaskan and Washington populations. *Le*: *L. epichlora*; *Lh*: *L. hexactis*; *La*: *L. aequalis*. Standard errors in parentheses. *N*: no. of individuals. Units of measurements as in Table 2

| | Alaska | | Washington | | |
|---|---|---|---|---|---|
| | *Le* | *Lh* | *Le* | *Lh* | *La* |
| Relative frequency | 0.85 (0.02) | 0.15 (0.02) | 0.49 (0.02) | 0.13 (0.02) | 0.38 (0.02) |
| Wet wt | 3.69 (0.09) | 11.85 (0.80) | 3.59 (0.16) | 4.18 (0.31) | 3.29 (0.16) |
| *R* | 2.27 (0.02) | 3.73 (0.12) | 2.24 (0.46) | 2.53 (0.09) | 2.29 (0.05) |
| *r* | 0.68 (0.01) | 1.01 (0.03) | 0.64 (0.01) | 0.70 (0.02) | 0.64 (0.01) |
| *R* ratio | 3.38 (0.03) | 3.69 (0.07) | 3.54 (0.05) | 3.68 (0.12) | 3.59 (0.05) |
| Frequency of males | 0.52 (0.02) | 0.47 (0.05) | 0.44 (0.03) | 0.49 (0.06) | 0.53 (0.04) |
| *N* | 490 | 87 | 225 | 62 | 176 |

*Leptasterias epichlora* and *L. hexactis* did not differ in either the placement or appearance of the abactinal, carinal, or superomarginal spines. *L. aequalis*, however, differed from the other two forms in that the carinal spines did not generally form well-defined rows, and there were one or two superomarginal spines on each plate. *L. epichlora* and *L. hexactis* always had one superomarginal spine per plate.

Variability in number of rays among the 1040 individuals examined included 1 *Leptasterias hexactis* with 4 rays, 3 *L. epichlora* with 5 rays, 3 *L. epichlora* with 7 rays, and 51 individuals with regenerating rays (20 *L. epichlora*, 18 *L. aequalis* and 13 *L. hexactis*). Additional phenotypic variation included 4 *L. epichlora* that were totally devoid of aboral pedicellariae and 1 *L. epichlora* that was colorless (white).

The relative frequency of occurrence of the species was determined from the collections for each state (Table 3). For both the Alaskan and Washington populations, *Leptasterias epichlora* was far more abundant than *L. hexactis*. *L. aequalis* had an intermediate frequency of occurrence in the Washington populations and was absent from all Alaskan populations. The sex ratio for each collection did not deviate significantly ($p > 0.05$) from 50:50 (Table 3).

The morphologies of all three forms were highly variable. Local variants existed and intergradation was common at some sites, especially San Juan Island, Washington. No single morphological character provided reliable separation between the forms. The general morphological features of *Leptasterias aequalis* were more variable than for either *L. epichlora* or *L. hexactis*, with features graded from "*L. epichlora*-like" to "*L. hexactis*-like". Differences in ray shape, coloration, markings and, perhaps most importantly, the arrangement and abundance of crossed minor pedicellariae on the aboral surface, provide the most reliable separation between *Leptasterias* spp. in Alaska and Washington. Voucher specimens of each species have been placed in the British Columbia Provincial Museum, Victoria, British Columbia, Canada.

## Electrophoretic analysis

### *Allele frequencies*

Allele frequencies, mean observed heterozygosity ($H_o$), expected (unbiased) heterozygosity ($H_e$), Weir and Cockerham's (1984) fixation index ($F_{IS}$), and sample size ($N$) for *Leptasterias* spp. are given in Table 4 (Alaskan samples) and Table 5 (Washington samples). Four of the 14 loci examined, *Est-1, Gpdh, Ipo-1* and *Pep-2*, were monomorphic (95% criterion) and fixed for the same allele in all species and populations examined, and are therefore not included in Tables 4–6.

For each species, the most common alleles were the same in both Alaskan and Washington populations, with the following exceptions: *Got* 108 was more common in all species at Lonesome Cove and Friday Harbor, Washington; *Idh* 120 occurred more frequently in *Leptasterias epichlora* at Eagle River and Auke Bay, Alaska; *Ipo-2* 144 was more common in all Washington populations, with the exception of *L. epichlora* at False Bay and Mitchell Bay, and was totally absent from Alaskan populations; *Ipo-2* 122 occurred more frequently in all Alaskan *L. hexactis* and was absent from all Washington populations except Lonesome Cove; *Pgi* 60 was more common in *L. hexactis* for all locations except Eagle River, Alaska, and False Bay and Mitchell Bay, Washington, and for all species at Lonesome Cove, Washington; and *Pgm* 84 was more common in *L. hexactis* at Lonesome Cove, Washington.

A number of fixed or nearly fixed allelic differences were observed between *Leptasterias epichlora* and *L. hexactis*. With the exception of *L. epichlora* at Lonesome Cove and Friday Harbor, Washington, *Pgi* 60 did not occur at frequencies greater than 0.02 for Washington *L. epichlora* and was absent from all Alaskan populations of *L. epichlora*, with the exception of one individual at Bridget Cove. Thus, *Pgi* 60 was diagnostic for *L. hexactis* in Alaskan samples, even though the more common *Pgi* 100 was shared by both species. Similarly, whereas *Ipo-2* 100 was shared in both *L. epichlora* and *L. hexactis* from Alaska, *Ipo-2* 122 did not occur in *L. epichlora* and was, therefore, diagnostic for *L. hexactis* in the Alaskan samples. Finally, the *Pep-1* locus was diagnostic between *L. epichlora* and *L. hexactis*. With no exceptions, *L. epichlora* in both Alaskan and Washington samples carried either the *Pep-1* 100, 95 or 90 alleles but never the faster migrating alleles (112, 107 and 103). Conversely, *L. hexactis* carried only the faster migrating *Pep-1* alleles, with the exception of some overlap at Friday Harbor, Washington, and Eagle River, Alaska.

### *Genetic variation*

For Weir and Cockerham's (1984) hierarchical *F*-statistics (Table 6), three levels of population structure were

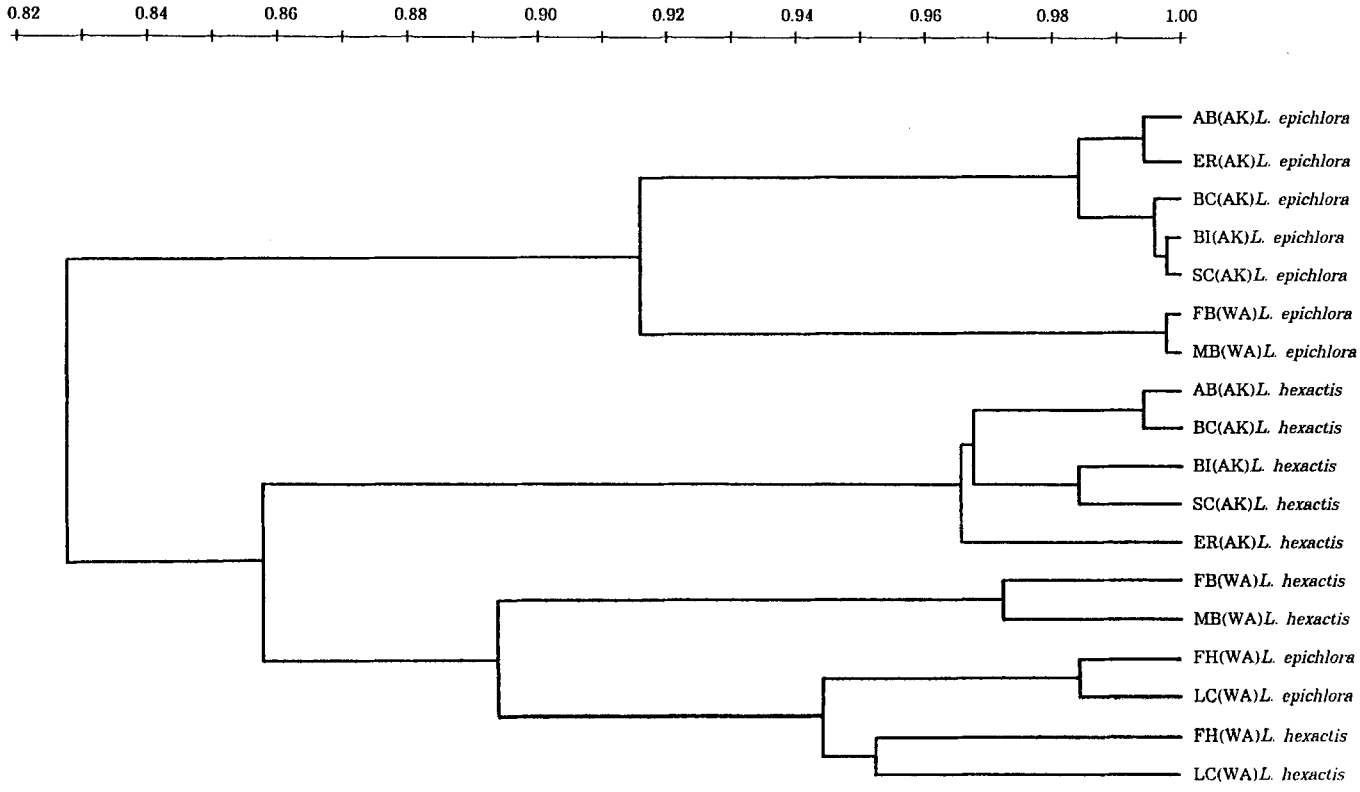


**Fig. 1.** *Leptasterias epichlora* and *L. hexactis*. UPGMA phenogram derived from Nei's genetic identity based on 14 loci. Site abbreviations are given in Table 7. AK: Alaska; WA: Washington

recognized within the species *Leptasterias epichlora* and *L. hexactis*. Since most population genetic models assume no gene flow between species and, perhaps most critically, a state of genetic and demographic equilibrium, the presumptive hybrid, *L. aequalis*, was omitted from this analysis. With the exception of two loci (*6-Pgd* and *Pgi*) in *L. epichlora*, there was a deficiency in the frequency of heterozygous individuals from Hardy-Weinberg proportions for both species, as reflected by positive $F_{ID}$ values. Mean weighted $F_{ID}$ values ($\pm$SE) summed across all polymorphic loci within a species were $0.124 \pm 0.042$ for *L. epichlora* and $0.311 \pm 0.080$ for *L. hexactis*, and were significantly ($p < 0.05$) greater than zero (Student's *t*-test) in both species.

There was considerable differentiation among demes within a region ($F_{DR}$). In all cases, high $F_{DR}$ values were due to allele frequency differences among demes rather than fixed allelic differences. Mean weighted $F_{DR}$ values ($\pm$SE) summed across all loci were $0.257 \pm 0.069$ for *Leptasterias epichlora* and $0.150 \pm 0.029$ for *L. hexactis*, and were significantly ($p < 0.05$) greater than zero (Student's *t*-test). Demes at Lonesome Cove and Friday Harbor, Washington, accounted for much of this variation, most notably at *Got, Ipo-2, Pgi* and *Pgm*.

$F_{RT}$ values (differentiation among states within the total area sampled) were heterogeneous for both *Leptasterias epichlora* and *L. hexactis*. High $F_{RT}$ values for *Ipo-2* in *L. hexactis* were due to a fixed allelic difference between Alaskan and Washington populations. In all other cases, positive $F_{RT}$ values reflect allele-frequency differences between states. Mean weighted $F_{RT}$ values ($\pm$SE) were $0.287 \pm 0.041$ for *L. epichlora* and $0.214 \pm 0.147$ for *L. hexactis*, indicating moderate differentiation between the Alaskan and Washington populations.

Estimates of genetic variability within populations of *Leptasterias* spp. are given in Table 7 in terms of the mean number of alleles per locus, percentage of polymorphic loci, and mean heterozygosity. Mean percentage of polymorphic loci summed across all populations within a species was 50.0 for *L. epichlora*, 64.3 for *L. hexactis* and 42.9 for *L. aequalis*. Mean observed heterozygosity ($\bar{H}_o \pm$SE) summed across all polymorphic loci and all individuals within a species were $0.092 \pm 0.030$, $0.135 \pm 0.036$, and $0.135 \pm 0.044$ for *L. epichlora*, *L. hexactis* and *L. aequalis*, respectively.

### *Phenetic relationships*

A UPGMA phenogram based on Nei's (1978) unbiased genetic identity (*I*) is presented in Fig. 1 for *Leptasterias epichlora* and *L. hexactis*. The presumptive hybrid, *L. aequalis*, was omitted from this analysis for the reasons stated in the preceding subsection. The first dichotomy occurred between the species *L. epichlora* and *L. hexactis* at an identity of 0.828 or distance of 0.189, with the exception of populations at Lonesome Cove and Friday Harbor. Genetic differentiation between *L. epichlora* and *L. hexactis* from Lonesome Cove and Friday Harbor was less than at all other locations examined. The second dichotomy occurred between states within each species at an identity of 0.916 ($D = 0.088$) for *L. epichlora* and 0.857

**Table 4.** *Lepasterias epichlora* (*Le*) and *L. hexactis* (*Lh*). Allele frequencies for 10 variable loci in 5 populations from Lynn Canal, Alaska. Alleles are expressed as mobility relative to most common allele (100). $H_o$, $H_e$: observed and expected (unbiased) frequencies of heterozygous individuals, respectively; $F_{ID}$: fixation index; $N$: no. of individuals sampled

| Locus and alleles | Bridget Cove | | Sunset Cove | | Benjamin Island | | Eagle River | | Auke Bay | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Le* | *Lh* | *Le* | *Lh* | *Le* | *Lh* | *Le* | *Lh* | *Le* | *Lh* |
| *Alkp* | | | | | | | | | | |
| 125 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 115 | 0.01 | 0.00 | 0.01 | 0.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| 108 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| 100 | 0.92 | 0.88 | 0.99 | 0.50 | 0.99 | 0.96 | 1.00 | 0.73 | 0.96 | 0.87 |
| 95 | 0.01 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 80 | 0.07 | 0.10 | 0.00 | 0.00 | 0.01 | 0.04 | 0.00 | 0.15 | 0.02 | 0.13 |
| $H_o$ | 0.14 | 0.23 | 0.02 | 1.00 | 0.01 | 0.07 | 0.00 | 0.31 | 0.04 | 0.00 |
| $H_e$ | 0.16 | 0.21 | 0.02 | 1.00 | 0.01 | 0.07 | 0.00 | 0.45 | 0.07 | 0.24 |
| $F_{ID}$ | 0.08 | −0.09 | −0.01 | 0.00 | −0.01 | −0.03 | −[a] | 0.31 | 0.49 | 1.00 |
| *Got* | | | | | | | | | | |
| 113 | 0.01 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.02 | 0.00 | 0.08 | 0.00 |
| 108 | 0.06 | 0.00 | 0.14 | 0.00 | 0.06 | 0.00 | 0.02 | 0.00 | 0.03 | 0.03 |
| 104 | 0.01 | 0.00 | 0.02 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 100 | 0.92 | 1.00 | 0.84 | 1.00 | 0.88 | 1.00 | 0.96 | 1.00 | 0.89 | 0.97 |
| 94 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.08 | 0.00 | 0.27 | 0.00 | 0.17 | 0.00 | 0.05 | 0.00 | 0.17 | 0.07 |
| $H_e$ | 0.15 | 0.00 | 0.27 | 0.00 | 0.22 | 0.00 | 0.08 | 0.00 | 0.21 | 0.07 |
| $F_{ID}$ | 0.43 | – | 0.02 | – | 0.22 | – | 0.36 | – | 0.18 | 0.00 |
| *Idh* | | | | | | | | | | |
| 131 | 0.06 | 0.15 | 0.03 | 0.00 | 0.08 | 0.04 | 0.03 | 0.00 | 0.06 | 0.10 |
| 120 | 0.33 | 0.27 | 0.31 | 0.00 | 0.15 | 0.15 | 0.52 | 0.00 | 0.45 | 0.23 |
| 111 | 0.04 | 0.02 | 0.03 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 |
| 100 | 0.46 | 0.56 | 0.61 | 1.00 | 0.61 | 0.74 | 0.11 | 1.00 | 0.33 | 0.67 |
| 94 | 0.11 | 0.00 | 0.02 | 0.00 | 0.12 | 0.07 | 0.02 | 0.00 | 0.12 | 0.00 |
| 86 | 0.00 | 0.00 | 0.01 | 0.00 | 0.04 | 0.00 | 0.31 | 0.00 | 0.03 | 0.00 |
| $H_o$ | 0.73 | 0.45 | 0.51 | 0.00 | 0.43 | 0.22 | 0.63 | 0.00 | 0.61 | 0.20 |
| $H_e$ | 0.67 | 0.59 | 0.54 | 0.00 | 0.58 | 0.43 | 0.62 | 0.00 | 0.67 | 0.51 |
| $F_{ID}$ | −0.09 | 0.24 | 0.05 | – | 0.26 | 0.48 | −0.02 | – | 0.08 | 0.61 |
| *Ipo-2* | | | | | | | | | | |
| 144 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 122 | 0.00 | 0.70 | 0.00 | 1.00 | 0.00 | 0.85 | 0.00 | 0.73 | 0.00 | 0.83 |
| 100 | 1.00 | 0.30 | 1.00 | 0.00 | 0.99 | 0.15 | 1.00 | 0.27 | 0.99 | 0.17 |
| 78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| $H_o$ | 0.00 | 0.42 | 0.00 | 0.00 | 0.02 | 0.30 | 0.00 | 0.38 | 0.01 | 0.07 |
| $H_e$ | 0.00 | 0.43 | 0.00 | 0.00 | 0.02 | 0.26 | 0.00 | 0.41 | 0.01 | 0.29 |
| $F_{ID}$ | – | 0.03 | – | – | −0.01 | −0.15 | – | 0.06 | 0.00 | 0.77 |
| *Mdh-1* | | | | | | | | | | |
| 116 | 0.00 | 0.08 | 0.01 | 0.00 | 0.00 | 0.39 | 0.00 | 0.08 | 0.00 | 0.30 |
| 100 | 0.96 | 0.92 | 0.93 | 1.00 | 0.90 | 0.61 | 0.95 | 0.92 | 0.99 | 0.70 |
| 90 | 0.04 | 0.00 | 0.06 | 0.00 | 0.10 | 0.00 | 0.05 | 0.00 | 0.01 | 0.00 |
| $H_o$ | 0.02 | 0.10 | 0.08 | 0.00 | 0.16 | 0.33 | 0.08 | 0.15 | 0.01 | 0.33 |
| $H_e$ | 0.07 | 0.15 | 0.13 | 0.00 | 0.18 | 0.48 | 0.09 | 0.15 | 0.01 | 0.43 |
| $F_{ID}$ | 0.66 | 0.36 | 0.34 | – | 0.14 | 0.31 | 0.19 | −0.04 | 0.00 | 0.23 |
| *Pep-1* | | | | | | | | | | |
| 112 | 0.00 | 0.43 | 0.00 | 0.00 | 0.00 | 0.24 | 0.00 | 0.12 | 0.00 | 0.37 |
| 107 | 0.00 | 0.57 | 0.00 | 1.00 | 0.00 | 0.76 | 0.00 | 0.46 | 0.00 | 0.63 |
| 103 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.15 | 0.00 | 0.00 |
| 100 | 0.95 | 0.00 | 0.82 | 0.00 | 0.88 | 0.00 | 0.98 | 0.27 | 0.96 | 0.00 |
| 95 | 0.05 | 0.00 | 0.18 | 0.00 | 0.12 | 0.00 | 0.02 | 0.00 | 0.04 | 0.00 |
| 90 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.08 | 0.36 | 0.28 | 0.00 | 0.08 | 0.11 | 0.01 | 0.54 | 0.09 | 0.20 |
| $H_e$ | 0.10 | 0.50 | 0.30 | 0.00 | 0.21 | 0.37 | 0.03 | 0.70 | 0.08 | 0.48 |
| $F_{ID}$ | 0.18 | 0.29 | 0.07 | – | 0.62 | 0.70 | 0.66 | 0.24 | −0.04 | 0.58 |

**Table 4** (continued)

| Locus and alleles | Bridget Cove | | Sunset Cove | | Benjamin Island | | Eagle River | | Auke Bay | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Le* | *Lh* | *Le* | *Lh* | *Le* | *Lh* | *Le* | *Lh* | *Le* | *Lh* |
| *6-Pgd* | | | | | | | | | | |
| 110 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 100 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 83 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_e$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $F_{ID}$ | – | – | – | – | 0.00 | – | – | – | – | – |
| *Pgi*[b] | | | | | | | | | | |
| 100 | 0.99 | 0.61 | 1.00 | 0.50 | 1.00 | 0.17 | 1.00 | 0.23 | 1.00 | 0.53 |
| 60 | 0.01 | 0.39 | 0.00 | 0.50 | 0.00 | 0.83 | 0.00 | 0.77 | 0.00 | 0.47 |
| $H_o$ | 0.01 | 0.52 | 0.00 | 1.00 | 0.00 | 0.33 | 0.00 | 0.15 | 0.00 | 0.53 |
| $H_e$ | 0.01 | 0.48 | 0.00 | 1.00 | 0.00 | 0.28 | 0.00 | 0.37 | 0.00 | 0.51 |
| $F_{ID}$ | 0.00 | −0.07 | – | 0.00 | – | −0.18 | – | 0.58 | – | −0.04 |
| *Pgm* | | | | | | | | | | |
| 116 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.11 | 0.00 | 0.00 | 0.02 | 0.20 |
| 105 | 0.00 | 0.02 | 0.01 | 0.50 | 0.00 | 0.33 | 0.01 | 0.12 | 0.00 | 0.10 |
| 100 | 1.00 | 0.90 | 0.98 | 0.00 | 1.00 | 0.56 | 0.99 | 0.88 | 0.97 | 0.70 |
| 92 | 0.00 | 0.00 | 0.00 | 0.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| 84 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 74 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.00 | 0.13 | 0.03 | 1.00 | 0.00 | 0.44 | 0.01 | 0.23 | 0.04 | 0.33 |
| $H_e$ | 0.00 | 0.18 | 0.03 | 1.00 | 0.00 | 0.58 | 0.01 | 0.21 | 0.06 | 0.48 |
| $F_{ID}$ | – | 0.28 | −0.01 | 0.00 | – | 0.23 | 0.00 | −0.09 | 0.32 | 0.30 |
| *Xdh* | | | | | | | | | | |
| 113 | 0.02 | 0.24 | 0.06 | 0.00 | 0.00 | 0.13 | 0.01 | 0.38 | 0.00 | 0.07 |
| 107 | 0.00 | 0.00 | 0.01 | 0.00 | 0.02 | 0.02 | 0.02 | 0.00 | 0.01 | 0.00 |
| 100 | 0.92 | 0.73 | 0.90 | 1.00 | 0.82 | 0.81 | 0.65 | 0.62 | 0.64 | 0.83 |
| 93 | 0.06 | 0.03 | 0.03 | 0.00 | 0.15 | 0.04 | 0.22 | 0.00 | 0.34 | 0.10 |
| 87 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.10 | 0.00 | 0.01 | 0.00 |
| $H_o$ | 0.12 | 0.10 | 0.11 | 0.00 | 0.19 | 0.19 | 0.43 | 0.00 | 0.41 | 0.07 |
| $H_e$ | 0.16 | 0.42 | 0.18 | 0.00 | 0.31 | 0.32 | 0.52 | 0.49 | 0.48 | 0.30 |
| $F_{ID}$ | 0.24 | 0.77 | 0.39 | – | 0.37 | 0.43 | 0.17 | 1.00 | 0.15 | 0.77 |
| *N* | 84 | 31 | 119 | 1 | 88 | 27 | 93 | 13 | 106 | 15 |

[a] $F_{ID}$ is undefined for a monomorphic locus
[b] Exhibited cathodal migration

($D=0.154$) for *L. hexactis*, with the exception of the Lonesome Cove and Friday Harbor populations. Further bifurcations occurred among individual demes within each state.

The results of the canonical discriminant analysis of *Leptasterias* spp. individuals are summarized in Fig. 2 for the Alaskan populations (A), Washington populations (B), and all populations combined (C). Based on the stepwise canonical discriminant analysis, separation between species was primarily based on *Pep-1* alleles. With the exception of Eagle River, where six individuals were heterozygous for *Pep-1* alleles that distinguish *L. epichlora* and *L. hexactis* (presumptive hybrids, although morphologically resembling *L. hexactis*), all individuals of each species fell within their respective mean 95% confidence intervals ($\bar{x} \pm 1.96$) for the Alaskan populations. A mean separation of 20.37 was obtained between *L. epichlora* and *L. hexactis* for Alaskan populations. For the Washington populations, nearly all *L. epichlora* and *L. hexactis* fell within their respective 95% confidence intervals, with a mean separation of 19.77. *L. aequalis*, in general, exhibited intermediate canonical values (overall mean = 10.46), although there was considerable overlap with the other species. Because separation between the *Leptasterias* species was primarily based on *Pep-1* alleles, *L. aequalis* individuals whose canonical scores overlapped with scores typical of either *L. epichlora* or *L. hexactis* carried *Pep-1* alleles characteristic of that species with which they overlapped. Because $F_1$ hybrids between *L. epichlora* and *L. hexactis* would necessarily be heterozygous at the *Pep-1* locus, carrying one allele characteristic of each parental species, individuals of *L. aequalis* whose scores overlapped with either of the parental species could not be $F_1$ progeny, but most likely represent advanced age segregants, i.e., hybrid intercrosses or backcrosses to the parental species.

**Table 5.** *Leptasterias epichlora* (*Le*), *L. hexactis* (*Lh*) and *L. aequalis* (*La*). Allele frequencies for 10 variable loci in 4 populations from San Juan Island, Washington. Abbreviations as in Table 4

| Locus and alleles | Lonesome Cove | | | Friday Harbor | | | False Bay | | | Mitchell Bay | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Le* | *Lh* | *La* | *Le* | *Lh* | *La* | *Le* | *Lh* | *La* | *Le* | *Lh* | *La* |
| *Alkp* | | | | | | | | | | | | |
| 125 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.02 | 0.22 | 0.05 | 0.00 | 0.00 | 0.00 |
| 115 | 0.00 | 0.00 | 0.00 | 0.02 | 0.03 | 0.07 | 0.01 | 0.03 | 0.05 | 0.03 | 0.00 | 0.03 |
| 108 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 100 | 1.00 | 1.00 | 1.00 | 0.96 | 0.97 | 0.87 | 0.97 | 0.75 | 0.90 | 0.96 | 1.00 | 0.97 |
| 95 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 80 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| $H_o$ | 0.00 | 0.00 | 0.00 | 0.02 | 0.07 | 0.14 | 0.03 | 0.38 | 0.19 | 0.02 | 0.00 | 0.03 |
| $H_e$ | 0.00 | 0.00 | 0.00 | 0.07 | 0.07 | 0.23 | 0.06 | 0.40 | 0.18 | 0.07 | 0.00 | 0.06 |
| $F_{ID}$ | – | – | – | 0.80 | 0.00 | 0.38 | 0.49 | 0.07 | −0.06 | 0.66 | – | 0.49 |
| *Got* | | | | | | | | | | | | |
| 113 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 |
| 108 | 0.81 | 0.84 | 0.81 | 0.74 | 0.60 | 0.68 | 0.00 | 0.06 | 0.15 | 0.06 | 0.20 | 0.18 |
| 104 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 100 | 0.19 | 0.16 | 0.19 | 0.26 | 0.40 | 0.29 | 1.00 | 0.94 | 0.85 | 0.94 | 0.80 | 0.81 |
| 94 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.26 | 0.31 | 0.30 | 0.30 | 0.27 | 0.36 | 0.00 | 0.13 | 0.10 | 0.07 | 0.27 | 0.25 |
| $H_e$ | 0.30 | 0.27 | 0.31 | 0.39 | 0.50 | 0.46 | 0.00 | 0.12 | 0.25 | 0.11 | 0.33 | 0.32 |
| $F_{ID}$ | 0.14 | −0.15 | 0.04 | 0.21 | 0.46 | 0.23 | – | −0.03 | 0.62 | 0.37 | 0.19 | 0.23 |
| *Idh* | | | | | | | | | | | | |
| 131 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.10 | 0.05 |
| 120 | 0.02 | 0.03 | 0.00 | 0.03 | 0.07 | 0.04 | 0.00 | 0.09 | 0.00 | 0.00 | 0.37 | 0.18 |
| 111 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.01 |
| 100 | 0.98 | 0.97 | 0.98 | 0.97 | 0.93 | 0.96 | 1.00 | 0.81 | 0.98 | 1.00 | 0.53 | 0.74 |
| 94 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 |
| 86 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.04 | 0.06 | 0.00 | 0.03 | 0.00 | 0.07 | 0.00 | 0.25 | 0.03 | 0.00 | 0.20 | 0.09 |
| $H_e$ | 0.04 | 0.06 | 0.04 | 0.03 | 0.13 | 0.07 | 0.00 | 0.34 | 0.03 | 0.00 | 0.59 | 0.41 |
| $F_{ID}$ | −0.01 | 0.00 | 1.00 | −0.01 | 1.00 | −0.02 | – | 0.26 | 0.00 | – | 0.66 | 0.79 |
| *Ipo-2* | | | | | | | | | | | | |
| 144 | 0.59 | 0.56 | 0.52 | 0.84 | 0.80 | 0.91 | 0.00 | 1.00 | 0.77 | 0.11 | 1.00 | 0.79 |
| 122 | 0.00 | 0.03 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 |
| 100 | 0.41 | 0.41 | 0.47 | 0.16 | 0.20 | 0.09 | 1.00 | 0.00 | 0.23 | 0.88 | 0.00 | 0.19 |
| 78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| $H_o$ | 0.56 | 0.50 | 0.42 | 0.23 | 0.40 | 0.18 | 0.00 | 0.00 | 0.32 | 0.07 | 0.00 | 0.12 |
| $H_e$ | 0.49 | 0.53 | 0.51 | 0.27 | 0.33 | 0.17 | 0.00 | 0.00 | 0.36 | 0.21 | 0.00 | 0.34 |
| $F_{ID}$ | −0.14 | 0.06 | 0.18 | 0.16 | −0.21 | −0.08 | – | – | 0.09 | 0.67 | – | 0.65 |
| *Mdh-1* | | | | | | | | | | | | |
| 116 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 100 | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 90 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_e$ | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $F_{ID}$ | – | – | 0.05 | – | – | – | – | – | – | – | – | – |
| *Pep-1* | | | | | | | | | | | | |
| 112 | 0.00 | 0.22 | 0.01 | 0.00 | 0.43 | 0.16 | 0.00 | 0.34 | 0.18 | 0.00 | 0.60 | 0.20 |
| 107 | 0.00 | 0.78 | 0.31 | 0.00 | 0.13 | 0.21 | 0.00 | 0.66 | 0.37 | 0.00 | 0.40 | 0.25 |
| 103 | 0.00 | 0.00 | 0.05 | 0.00 | 0.33 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 |
| 100 | 0.72 | 0.00 | 0.49 | 0.85 | 0.01 | 0.43 | 0.00 | 0.00 | 0.10 | 0.08 | 0.00 | 0.20 |
| 95 | 0.28 | 0.00 | 0.14 | 0.14 | 0.00 | 0.18 | 1.00 | 0.00 | 0.35 | 0.91 | 0.33 | 0.00 |
| 90 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| $H_o$ | 0.37 | 0.19 | 0.60 | 0.26 | 0.07 | 0.75 | 0.00 | 0.19 | 0.32 | 0.05 | 0.27 | 0.34 |
| $H_e$ | 0.41 | 0.35 | 0.65 | 0.25 | 0.70 | 0.73 | 0.00 | 0.47 | 0.71 | 0.18 | 0.50 | 0.75 |
| $F_{ID}$ | 0.09 | 0.47 | 0.07 | −0.03 | 0.61 | −0.03 | – | 0.60 | 0.54 | 0.73 | 0.46 | 0.55 |

**Table 5** (continued)

| Locus and alleles | Lonesome Cove | | | Friday Harbor | | | False Bay | | | Mitchell Bay | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Le* | *Lh* | *La* | *Le* | *Lh* | *La* | *Le* | *Lh* | *La* | *Le* | *Lh* | *La* |
| *6-Pgd* | | | | | | | | | | | | |
| 110 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 100 | 0.99 | 1.00 | 0.96 | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 83 | 0.01 | 0.00 | 0.04 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.02 | 0.00 | 0.08 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_e$ | 0.02 | 0.00 | 0.08 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $F_{ID}$ | 0.00 | – | −0.03 | – | – | 0.00 | – | – | – | – | – | – |
| *Pgi* | | | | | | | | | | | | |
| 100 | 0.30 | 0.28 | 0.32 | 0.64 | 0.70 | 0.55 | 0.98 | 0.63 | 0.81 | 0.99 | 0.20 | 0.83 |
| 60 | 0.70 | 0.72 | 0.68 | 0.36 | 0.30 | 0.45 | 0.02 | 0.37 | 0.19 | 0.01 | 0.80 | 0.17 |
| $H_o$ | 0.48 | 0.31 | 0.48 | 0.50 | 0.33 | 0.54 | 0.00 | 0.38 | 0.26 | 0.02 | 0.27 | 0.21 |
| $H_e$ | 0.42 | 0.42 | 0.44 | 0.46 | 0.43 | 0.50 | 0.03 | 0.48 | 0.32 | 0.02 | 0.33 | 0.29 |
| $F_{ID}$ | −0.14 | 0.25 | −0.09 | −0.08 | 0.23 | −0.06 | 1.00 | 0.23 | 0.19 | 0.00 | 0.19 | 0.30 |
| *Pgm* | | | | | | | | | | | | |
| 116 | 0.27 | 0.16 | 0.24 | 0.23 | 0.13 | 0.16 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.10 |
| 105 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 |
| 100 | 0.33 | 0.31 | 0.41 | 0.37 | 0.43 | 0.50 | 0.86 | 0.94 | 0.95 | 0.96 | 0.94 | 0.80 |
| 92 | 0.14 | 0.16 | 0.21 | 0.14 | 0.13 | 0.05 | 0.14 | 0.06 | 0.03 | 0.02 | 0.03 | 0.01 |
| 84 | 0.21 | 0.37 | 0.14 | 0.26 | 0.30 | 0.29 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.09 |
| 74 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.69 | 0.75 | 0.58 | 0.82 | 0.53 | 0.57 | 0.22 | 0.13 | 0.10 | 0.07 | 0.13 | 0.25 |
| $H_e$ | 0.76 | 0.74 | 0.72 | 0.73 | 0.71 | 0.65 | 0.25 | 0.12 | 0.10 | 0.07 | 0.13 | 0.35 |
| $F_{ID}$ | 0.10 | −0.02 | 0.19 | −0.12 | 0.25 | 0.12 | 0.10 | −0.03 | −0.02 | −0.02 | −0.02 | 0.31 |
| *Xdh* | | | | | | | | | | | | |
| 113 | 0.00 | 0.00 | 0.00 | 0.00 | 0.07 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 107 | 0.00 | 0.03 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 100 | 0.91 | 0.97 | 0.95 | 0.96 | 0.76 | 0.94 | 0.98 | 1.00 | 0.98 | 0.99 | 1.00 | 0.98 |
| 93 | 0.09 | 0.00 | 0.04 | 0.02 | 0.17 | 0.04 | 0.02 | 0.00 | 0.02 | 0.01 | 0.00 | 0.02 |
| 87 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $H_o$ | 0.15 | 0.06 | 0.06 | 0.05 | 0.07 | 0.11 | 0.03 | 0.00 | 0.03 | 0.02 | 0.00 | 0.03 |
| $H_e$ | 0.17 | 0.06 | 0.10 | 0.07 | 0.39 | 0.10 | 0.03 | 0.00 | 0.03 | 0.02 | 0.00 | 0.03 |
| $F_{ID}$ | 0.13 | 0.00 | 0.38 | 0.39 | 0.83 | −0.02 | −0.01 | – | 0.00 | 0.00 | – | −0.01 |
| *N* | 54 | 16 | 50 | 66 | 15 | 28 | 63 | 16 | 31 | 42 | 15 | 67 |

**Table 6.** *Leptasterias epichlora* and *L. hexactis*. *F*-statistics. $F_{ID}$: genetic differentiation among individuals within a deme (a measure of departure of genotypic frequencies from Hardy-Weinberg expectations). $F_{DR}$: differentiation among demes within a region (state). $F_{RT}$: differentiation among regions within total area sampled. Standard errors in parentheses. *F*-statistics are undefined for a monomorphic locus

| Locus | *L. epichlora* | | | *L. hexactis* | | |
|---|---|---|---|---|---|---|
| | $F_{ID}$ | $F_{DR}$ | $F_{RT}$ | $F_{ID}$ | $F_{DR}$ | $F_{RT}$ |
| *Alkp* | 0.356 | 0.023 | −0.006 | 0.209 | 0.071 | 0.012 |
| *Got* | 0.190 | 0.373 | 0.219 | 0.188 | 0.450 | 0.379 |
| *Idh* | 0.054 | 0.114 | 0.302 | 0.444 | 0.094 | −0.013 |
| *Ipo-2* | 0.089 | 0.564 | 0.444 | 0.060 | 0.104 | 0.654 |
| *Mdh* | 0.275 | 0.019 | 0.030 | 0.283 | 0.125 | 0.156 |
| *Pep-1* | 0.201 | 0.427 | 0.397 | 0.485 | 0.116 | −0.019 |
| *6-Pgd* | −0.001 | 0.001 | −0.001 | – | – | – |
| *Pgi* | −0.069 | 0.422 | 0.300 | 0.101 | 0.191 | −0.054 |
| *Pgm* | 0.010 | 0.199 | 0.286 | 0.168 | 0.184 | 0.013 |
| *Xdh* | 0.230 | 0.093 | 0.058 | 0.728 | 0.032 | 0.087 |
| Mean | 0.124 (0.042) | 0.257 (0.069) | 0.287 (0.041) | 0.311 (0.080) | 0.150 (0.029) | 0.214 (0.147) |

**Table 7.** *Leptasterias* spp. Estimates of genetic variability in 9 populations based on 14 loci. A locus was considered to be polymorphic if frequency of most common allele did not exceed 0.95. Expected heterozygosities are unbiased estimates (Nei 1978). Standard errors in parentheses. Site abbreviations are used in Fig. 1

| Population (latitude; longitude) | *N* | Mean no. of alleles per locus | % of loci polymorphic | Mean heterozygosity | |
|---|---|---|---|---|---|
| | | | | observed | expected |
| Alaska (AK) | | | | | |
| Bridget Cove (BC) (58°38′N; 135°56′W) | | | | | |
| *L. epichlora* | 84 | 2.1 (0.4) | 35.7 | 0.085 (0.051) | 0.094 (0.047) |
| *L. hexactis* | 31 | 1.9 (0.3) | 57.1 | 0.164 (0.052) | 0.212 (0.061) |
| Sunset Cove (SC) (58°36′N; 135°56′W) | | | | | |
| *L. epichlora* | 119 | 2.1 (0.4) | 35.7 | 0.092 (0.041) | 0.104 (0.044) |
| *L. hexactis* | 1 | 1.2 (0.1) | 21.4 | 0.214 (0.114) | 0.214 (0.114) |
| Benjamin Island (BI) (58°33′N; 135°54′W) | | | | | |
| *L. epichlora* | 88 | 2.1 (0.4) | 35.7 | 0.077 (0.033) | 0.111 (0.046) |
| *L. hexactis* | 27 | 1.9 (0.3) | 50.0 | 0.143 (0.042) | 0.200 (0.057) |
| Eagle River (ER) (58°33′N; 135°51′W) | | | | | |
| *L. epichlora* | 93 | 2.0 (0.4) | 14.3 | 0.087 (0.052) | 0.097 (0.054) |
| *L. hexactis* | 13 | 1.7 (0.2) | 50.0 | 0.126 (0.047) | 0.199 (0.064) |
| Auke Bay (AB) (58°23′N; 135°42′W) | | | | | |
| *L. epichlora* | 106 | 2.2 (0.4) | 21.4 | 0.098 (0.050) | 0.133 (0.055) |
| *L. hexactis* | 15 | 1.9 (0.2) | 57.1 | 0.129 (0.045) | 0.236 (0.058) |
| Washington (WA) | | | | | |
| Lonesome Cove (LC), (48°37′N; 123°06′W) | | | | | |
| *L. epichlora* | 54 | 1.9 (0.3) | 42.9 | 0.183 (0.065) | 0.186 (0.066) |
| *L. hexactis* | 16 | 1.7 (0.2) | 35.7 | 0.156 (0.062) | 0.174 (0.066) |
| *L. aequalis* | 50 | 2.2 (0.3) | 42.9 | 0.181 (0.064) | 0.206 (0.071) |
| Friday Harbor (FH) (48°33′N; 123°01′W) | | | | | |
| *L. epichlora* | 66 | 1.9 (0.3) | 35.7 | 0.157 (0.066) | 0.162 (0.061) |
| *L. hexactis* | 15 | 1.9 (0.3) | 50.0 | 0.138 (0.049) | 0.233 (0.072) |
| *L. aequalis* | 28 | 2.3 (0.4) | 50.0 | 0.196 (0.068) | 0.211 (0.070) |
| False Bay (FB) (48°29′N; 123°04′W) | | | | | |
| *L. epichlora* | 63 | 1.4 (0.2) | 7.1 | 0.020 (0.016) | 0.027 (0.018) |
| *L. hexactis* | 16 | 1.7 (0.3) | 41.9 | 0.103 (0.038) | 0.138 (0.052) |
| *L. aequalis* | 31 | 1.9 (0.3) | 35.7 | 0.097 (0.033) | 0.141 (0.055) |

**Table 7** (continued)

| Population (latitude; longitude) | *N* | Mean no. of alleles per locus | % of loci polymorphic | Mean heterozygosity | |
|---|---|---|---|---|---|
| | | | | observed | expected |
| Mitchell Bay (MB) (48°34'N; 123°10'W) | | | | | |
| *L. epichlora* | 42 | 1.8 (0.2) | 21.4 | 0.024 (0.008) | 0.049 (0.019) |
| *L. hexactis* | 15 | 1.5 (0.2) | 35.7 | 0.081 (0.031) | 0.134 (0.056) |
| *L. aequalis* | 67 | 2.3 (0.4) | 42.9 | 0.092 (0.031) | 0.181 (0.056) |
| Summary | | | | | |
| *L. epichlora* | 715 | 3.1 (0.5) | 50.0 | 0.092 (0.030) | 0.164 (0.047) |
| *L. hexactis* | 149 | 2.8 (0.5) | 64.3 | 0.135 (0.036) | 0.254 (0.062) |
| *L. aequalis* | 176 | 2.9 (0.0) | 42.9 | 0.135 (0.044) | 0.216 (0.068) |

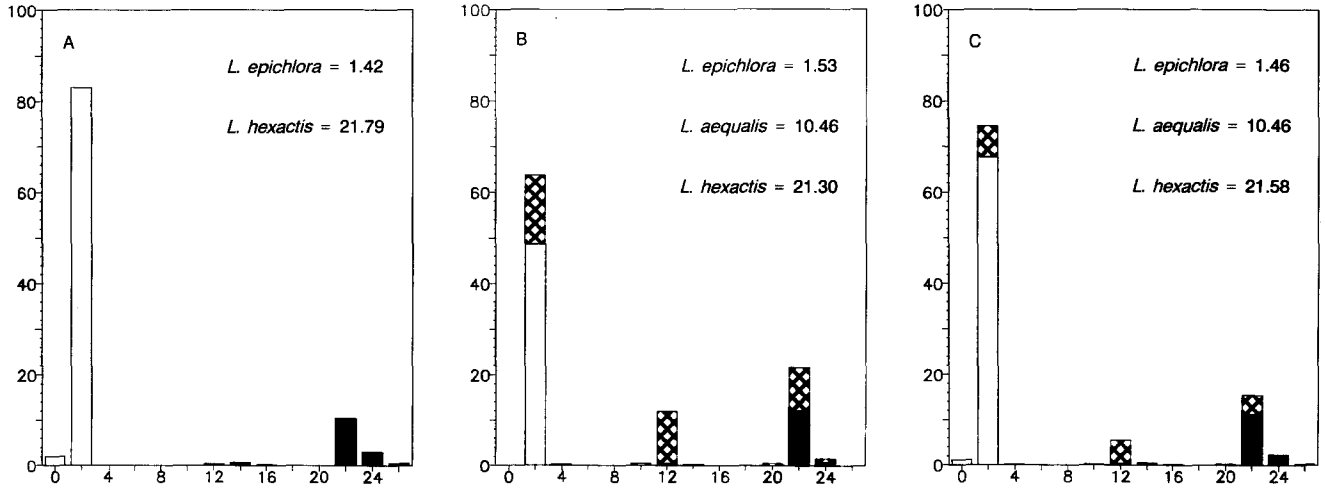


**Fig. 2.** *Leptasterias epichlora, L. hexactis* and *L. aequalis*. Distribution of individual canonical scores for Alaskan populations (A), Washington populations (B), and all populations combined (C). Canonical scores are given on abscissa, relative percentages on ordinate. Open bars represent *L. epichlora;* filled bars, *L. hexactis;* crosshatched bars, *L. aequalis*. 95% confidence intervals for each species (not indicated graphically) are $\bar{x} \pm Z_{(\alpha=0.05)} \cdot \sigma$, where $\bar{x}$ = mean canonical value for each respective species, $Z_{(\alpha=0.05)} = 95$ th percentile of the standard normal distribution (upper right of each graph), and $\sigma$ = standard deviation

## Discussion

Morphological examinations revealed two distinct forms of *Leptasterias* in samples from Lynn Canal, Alaska, *L. epichlora* (Brandt, 1835) *sensu* Verrill (1914) and *L. hexactis* (Stimpson, 1862a) *sensu* Verrill (1914), and three distinct forms from San Juan Island, Washington, *L. epichlora, L. hexactis*, and *L. aequalis* (Stimpson, 1862b) *sensu* Fisher (1930). In the latter region, the distinction between each of these forms was less clear, with no single morphological character being completely reliable in distinguishing *Leptasterias* spp. Based on both morphological and allozymic data, however, *L. epichlora* from Alaska and Washington are conspecific, as is *L. hexactis* from these two localities although minor morphological differences existed within species between the two states. The third form, *L. aequalis*, was only present in Washington, had a more variable appearance than either *L. epichlora* or *L. hexactis*, and was intermediate in many ways between these species.

*Leptasterias epichlora* and *L. hexactis* from Alaska and Washington were genetically distinct. There were fixed or nearly fixed allelic differences between these species at several loci (*Ipo-2, Pep-1* and *Pgi*), as well as other less pronounced allele-frequency differences. The genetic distance ($D = 0.19 \pm 0.01$) between these species is similar to that reported for other congeneric marine invertebrates (Ayala 1982).

The canonical discriminant analysis divided *Leptasterias* spp. individuals into three clusters. One cluster consisted largely of *L. epichlora* from both Alaska and Washington. Another cluster was largely composed of *L. hex-*

*actis* from both localities. The middle cluster contained individuals that were heterozygous for alleles, primarily *Pep-1* alleles, that distinguished *L. epichlora* and *L. hexactis*, and consisted almost exclusively of *L. aequalis*. These results do not lead to the rejection of our *a priori* hypothesis that *L. aequalis* is genetically intermediate to *L. epichlora* and *L. hexactis* and, in fact, suggest that *L. aequalis* is a hybrid between these species. The amount of hybridization varies between locations, with extensive hybridization occurring in the Puget Sound region and little or no evidence of hybridization in Alaska. Considerable genetic integrity, however, is maintained between *L. epichlora* and *L. hexactis* despite their apparent hybridization. The existence of individuals that resemble *L. aequalis* morphologically but have canonical scores characteristic of either *L. epichlora* or *L. hexactis* is probably best explained by the occurrence of advanced age segregants in the Washington samples.

Our data suggest that some gene flow occurs between *Leptasterias epichlora* and *L. hexactis*, particularly at Lonesome Cove and Friday Harbor, Washington. With increasing introgression, genetic variation within populations of a given species generally increases and variation between species generally decreases. The phenogram indicates that *L. epichlora* and *L. hexactis* at Lonesome Cove and Friday Harbor are genetically more similar than are these species at the other locations examined. In addition, mean observed heterozygosities for *L. epichlora* at these sites are considerably higher than at all other locations examined. If backcrosses of the presumptive hybrid, *L. aequalis*, to *L. epichlora* occur more frequently than backcrosses to *L. hexactis*, this asymmetry could explain the higher observed heterozygosities for *L. epichlora* and the higher degree of genetic similarity of *L. epichlora* to *L. hexactis* at Lonesome Cove and Friday Harbor. Cross-fertilization experiments and genetic analyses of natural populations may clarify the reproductive barriers between these species.

Weir and Cockerham's (1984) hierarchical *F*-statistics are useful in assessing the degree of genetic differentiation in subdivided populations. *Leptasterias epichlora* exhibited moderate differentiation among demes within each state ($F_{DR}$), while *L. hexactis* exhibited somewhat lower differentiation. Female *Leptasterias* spp. externally brood their young to a crawl-away juvenile stage, so their dispersal capability would appear to be low, although occasional rafting on macrophytes and other floating objects could potentially transport them considerable distances (Highsmith 1985). Without comparable data for sea stars exhibiting planktonic larval forms capable of long-distance dispersal, however, it is difficult to attribute these high $F_{DR}$ values to limited dispersal capability leading to a high degree of genetic differentiation among local populations. Many exceptions to the apparent inverse correlation between dispersal capability and the degree of genetic differentiation are known in marine invertebrates (Burton 1983, Hedgecock 1986) and may result from factors such as environmental heterogeneity and differential selection.

$F_{DT}$ measures the total genetic differentiation within a species and is comparable to $F_{ST}$ values from some other studies in which small-scale differentiation was not included. For example, Nishida and Lucas (1988) examined widely separated populations of the crown-of-thorns sea star *Acanthaster planci* throughout the Pacific basin, and reported $F_{ST}$ values ranging from 0.011 to 0.072 for distances of 400 to 7 500 km, respectively. Similarly, Nash et al. (1988) reported a value of 0.019 for populations of *A. planci* separated by a distance of ca. 1 300 km. Based on these small $F_{ST}$ values, Nishida and Lucas concluded that populations of *A. planci* throughout the Pacific basin are panmictic as a consequence of long-distance larval dispersal. In the present study, a distance of ca. 1 500 km separated collection sites in Alaska and Washington. Calculated $F_{DT}$ values for *Leptasterias epichlora* ($0.471 \pm 0.070$) and *L. hexactis* ($0.332 \pm 0.126$) were considerably larger than those reported for *A. planci*, indicating a substantially higher degree of genetic differentiation for the *Leptasterias* species. This high degree of genetic differentiation between populations of *L. epichlora* and *L. hexactis* may be due in part to restricted gene flow, a consequence of their limited dispersal capability. Populations of both species from Alaska and Washington are probably not panmictic.

Heterozygote deficiencies have been reported for a number of marine invertebrate species (Berger 1973, Ayala et al. 1973, Koehn et al. 1976), and many explanations for this condition have been offered (see Berger 1983, Zouros and Foltz 1984). There was a significant ($p < 0.05$) deficiency in the frequency of heterozygous individuals for both *Leptasterias epichlora* and *L. hexactis*, as reflected by positive $F_{ID}$ values. Because positive $F_{ID}$ values were found in both Alaskan and Washington populations of these species, and because *L. aequalis* was omitted from this analysis, the heterozygote deficiencies are not likely to be the result of hybridization. Mean observed heterozygosity for each species fell within the range reported for other species of sea stars (ca. 0.04 to 0.37).

The genetic distinctness of *Leptasterias epichlora* and *L. hexactis* in samples from Alaska indicates that they are distinct species in the biological sense, since no genetic exchange occurs between these forms, with the possible exception of populations at Eagle River. Extensive hybridization and genetic exchange, however, apparently occur between these species at San Juan Island. Do these forms, then, warrant the rank of full species? While it is not unprecedented to recognize taxa that hybridize within narrow zones where their ranges overlap as distinct species (e.g. McDonald and Koehn 1988, Dillon and Manzi 1989), it is dependent on the adoption of a modified species concept, such as the evolutionary species concept. In short, an evolutionary species has a lineage which maintains its integrity from other such lineages and has its own evolutionary tendencies, yet its definition is not dependent on the exclusive use of the interbreeding criterion as is a species defined by the traditional biological species concept (see Woodruff et al. 1988). We believe that the genetic integrity maintained between *L. epichlora* and *L. hexactis* in Alaska warrants their recognition as distinct evolutionary species despite their apparent hybridization in Washington. We suggest that the name

*L. epichlora* (Brandt, 1835) *sensu* Verrill (1914) be revived and used for the most common species found in both southeastern Alaska and the Puget Sound region, and the name *L. hexactis* (Stimpson, 1862a) *sensu* Verrill (1914) be reserved for what Fisher (1930) described as *L. hexactis* f. *hexactis*. Our data suggest that *L. aequalis* (Stimpson, 1862b) *sensu* Fisher (1930) in the Puget Sound region is a hybrid between *L. epichlora* and *L. hexactis*. The taxonomic status of *L. aequalis* outside the areas studied and the relationship of these taxa to other described species of *Leptasterias*, including *L. pusilla* Fisher, 1930 and *L. polaris* (Müller and Troschel, 1842), remains uncertain.

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## Literature cited

Ayala, F. J. (1982). Population and evolutionary genetics. Benjamin/Cummings, Menlo Park, California

Ayala, F. J., Hedgecock, D., Zumwalt, G. S., Valentine, J. W. (1973). Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. Evolution, Lawrence, Kansas 27: 177–191

Berger, E. M. (1973). Gene-enzyme variation in three sympatric species of *Littorina*. Biol. Bull. mar. biol. Lab., Woods Hole 145: 83–90

Berger, E. M. (1983). Population genetics of marine gastropods and bivalves In: Russell-Hunter, W. D. (ed.) The Mollusca. Vol. 6. Ecology. Academic Press, New York, p. 563–596

Brandt, (1835). Prodromus descriptionis. Animalium Abh., Mertensio Observatorum. Fascic. I. (polypos, acalephas discophoras et siphonophoras nec non Echinodermata contineus). p. 270 [cited after Verrill (1914)]

Burton, R. S. (1983). Protein polymorphisms and genetic differentiation of marine invertebrate populations. Mar. Biol. Lett. 4: 193–206

Bush, M. (1918). Key to the echinoderms of Friday Harbor, Washington. Publs Puget Sound mar. Biol. Stn 2: 17–44

Bush, M. (1921). Revised key to the echinoderms of Friday Harbor, Washington. Publs Puget Sound mar. Biol. Stn. 3: 65–77

Chambers, S. M. (1978). An electrophoretically detected sibling species of *"Goniobasis floridensis"* (Mesogastropoda: Pleuroceridae). Malacologia 178: 157–162

Chia, F.-S. (1964). The developmental and reproductive biology of a brooding starfish, *Leptasterias hexactis*. Ph. D. dissertation. University of Washington

Chia, F.-S. (1966). Systematics of the six-rayed sea star, *Leptasterias*, in the vicinity of San Juan Island, Washington. Syst. Zool. 15: 300–306

Dillon, R. T. Jr., Manzi, J. J. (1989). Genetics and shell morphology in a hybrid zone between the hard clams *Mercenaria mercenaria* and *M. campechiensis*. Mar. Biol. 100: 217–222

Fisher, W. K. (1930). Asteroidea of the North Pacific and adjacent waters. Part 3. Forcipulata. Bull. U.S. natn. Mus. 76: 1–255

Harris, H., Hopkinson, D.A. (1976). Handbook of enzyme electrophoresis in human genetics. North-Holland, Amsterdam

Hedgecock, D. (1986). Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bull. mar. Sci. 39: 550–564

Highsmith, R. C. (1985). Floating and algal rafting as potential dispersal mechanisms in brooding invertebrates. Mar. Ecol. Prog. Ser. 25: 169–179

Koehn, R. K., Milkman, R., Mitton, J. B. (1976). Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis*. Evolution, Lawrence, Kansas 30: 2–32

Kwast, K. E. (1989). Genetics and systematics of the *Leptasterias hexactis* species complex (Echinodermata: Asteroidea). M.S. thesis. Louisiana State University

Lambert, P. (1981). The sea stars of British Columbia. British Columbia Provincial Museum, Victoria

Levene, H. (1949). On a matching problem arising in genetics. Ann. math. Statist. 20: 91–94

Manwell, C., Baker, C. M. A. (1963). A sibling species of sea-cucumber discovered by starch gel electrophoresis. Comp. Biochem. Physiol. 10: 39–53

McDonald, J. H., Koehn, R. K. (1988). The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. Mar. Biol. 99: 111–118

Nash, W. J., Goddard, M., Lucas, J. S. (1988). Population genetics of the crown-of-thorns starfish, *Acanthaster planci* (L.), in the Great Barrier Reef region. Coral Reefs 7: 11–18

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, Austin, Tex. 89: 583–590

Nishida, M., Lucas, J. S. (1988). Genetic differences between geographic populations of the crown-of-thorns starfish throughout the Pacific region. Mar. Biol. 98: 359–368

SAS Institute, Inc. (1985). SAS user's guide: statistics. Version 5 ed. SAS Institute Inc., Cary, North Carolina

Schopf, T. J. M., Murphy, L. S. (1973). Protein polymorphism of the hybridizing seastars *Asterias forbesi* and *Asterias vulgaris* and implications for their evolution. Biol. Bull. mar. biol. Lab., Woods Hole 145: 589–597

Selander, R. K., Smith, M. H., Yang, S. Y., Johnson, W. E., Gentry, J. B. (1971). Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud. Genet., Austin, Tex. 6: 49–90

Smouse, P. E. (1972). The canonical analysis of multiple species hybridization. Biometrics 28: 361–371

Sneath, P. H. A., Sokal, R. R. (1973). Numerical taxonomy – the principles and practice of numerical classification. W. H. Freeman & Co., San Francisco

Steiner, W. W. M., Lisowski, E. A., Ostebur, D. (1977). Biochemical differences in sympatric colour morphs of an aquatic isopod (*Asellus brevicauda*). Comp. Biochem. Physiol. 56B: 371–374

Stimpson, W. (1862a). On new genera and species of starfishes of the family Pycnopodidae (*Asteracanthion*, Müll. and Trosch.): *Asterias hexactis*. Proc. Boston Soc. nat. Hist. 8: p. 272

Stimpson, W. (1862b). On new genera and species of starfishes of the family Pycnopodidae (*Asteracanthion*, Müll. and Trosch.): *Asterias aequalis*. Proc. Boston Soc. nat. Hist. 8: p. 273

Thorpe, J. P., Beardmore, J. A., Ryland, J. S. (1978). Genetic evidence for cryptic speciation in the marine bryozoan *Alcyonidium gelatinosum*. Mar. Biol. 49: 27–32

Tuttle, R. D., Lindahl, R. (1980). Genetic variability in 3 co-occurring forms of the starfish genus *Othilia*(=*Echinaster*). Experientia 36: 923–925

Verrill, W. K. (1914). Monograph of the shallow-water starfishes of the North Pacific coast from the Arctic Ocean to California. Harriman Alaska Exped. (Smithson. Instn) 14: 1–408

Weir, B. S., Cockerham, C.C. (1984). Estimating *F*-statistics for the analysis of population structure. Evolution, Lawrence, Kansas 38: 1358–1370

Woodruff, D. S., Staub, K. C., Upatham, E. S., Viyanant, V., Yuan, H.-C. (1988). Genetic variation in *Oncomelania hupensis: Schistosoma japonicum* transmitting snails in China and the Philippines are distinct species. Malacologia 29: 347–361

Zouros, E., Foltz, D. W. (1984). Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia 25: 583–591