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Population Genetics and Pattern of Larval Dispersal of the Endemic Hawaiian Freshwater Amphidromous Gastropod *Neritina granosa* (Prosobranchia: Neritidae)[1]

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ABSTRACT: Protein electrophoresis was used to study the population genetics of the endemic Hawaiian freshwater amphidromous gastropod *Neritina granosa* Sowerby. The genetic information was used to infer the pattern and degree of planktonic larval dispersal. Samples were taken from 12 streams located throughout the Hawaiian Archipelago during July, August, and September 1991. Overall mean heterozygosity was 0.052. Heterozygote deficiency was comparable with that found in other mollusks and marine invertebrates. Gene flow was substantial and was generally sufficient to maintain similar allele frequencies among stream populations. An island model of migration was indicated. However, significant heterogeneity among populations was observed and was due primarily to three geographically disparate streams. Causes of deficiency and heterogeneity remain unknown. Demographic information suggests that, although high from a genetic point of view, the rate of migration calculated from gene flow might be insufficient to affect demographic processes in large populations of *N. granosa.*

PRISTINE HAWAIIAN STREAMS host a native amphidromous fauna composed of endemic crustaceans, gobies, and gastropods (including *Neritina granosa* Sowerby) (Ford and Kinzie 1982, Kinzie and Ford 1982, Kinzie 1988, Anonymous 1990). All of these species are considered "freshwater amphidromous" (sensu McDowall 1992). Freshwater amphidromy is a subcategory of diadromy, whereby individuals live out the adult phase of their life cycle, including breeding, within a freshwater stream. The larvae produced are carried by the stream waters to the ocean where they develop as part of the marine plankton. Settlement-age larvae leave the ocean and return to the stream habitat to develop into adults. Adults do not migrate between streams. The only method of dispersal between streams is via the larval phase. Thus, in the Hawaiian freshwater amphidromous fauna, each stream population is a discrete entity. The stream populations are linked by some degree of larval dispersal to form a metapopulation.

This fauna is an important component of Hawai'i's biodiversity. It was used for subsistence in ancient times by Native Hawaiians. Today, certain species within this fauna continue to be valued by Native Hawaiians and Hawai'i's rural residents as a source of subsistence food items and recreation. Unfortunately, Hawai'i's native stream fauna has declined during historical times. Primary causes are habitat degradation (Maciolek 1975, 1978, Parrish et al. 1978, Ford and Yuen 1988) and extensive invasion by alien species (Maciolek 1975, 1984, Kinzie and Ford 1977, 1982, Timbol et al. 1980, Kinzie 1988, 1992). Despite their value, compara-

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tively little is known of the population genetics of these species. Practically nothing is known of the pattern and degree of dispersal of their planktonic larvae.

Understanding the connectivity of populations within a metapopulation is a key challenge in aquatic science (Doherty 1991). Likewise, information on larval dispersal is integral to conservation planning for Hawai'i's stream fauna (Ford and Yuen 1988, Anonymous 1990, Fairweather 1991). Knowledge of both the pattern and degree of dispersal is necessary for identification of the sources of recruits and the size and location of management units (e.g., Dizon et al. 1992). Persistence of the metapopulation itself depends on dispersal (Gilpin 1987). Where size and numbers of populations have been reduced, information on dispersal is critical to predicting persistence of individual populations and the metapopulation. (Reviews of metapopulation theory are provided by Kareiva [1990] and Hanski and Gilpin [1991].) However, the degree to which the larvae of Hawai'i's native stream fauna return to their natal stream or recruit to nonnatal streams is unknown for any of the species.

Direct measurement of larval dispersal is notoriously difficult (Leis 1991, Grosberg and Levitan 1992). Genetic studies have been used regularly with other fauna to infer the spatial pattern of dispersal among populations and to calculate the number of migrants per generation (Waples 1987).

Previous work on the population genetics of the Hawaiian amphidromous fauna was undertaken by Kinzie and Ford (1982) (*N. granosa*) and Fitzsimons et al. (1990) (the goby *Lentipes concolor*). Both studies concluded that gene flow is sufficient to prevent genetic divergence among streams (= populations) throughout Hawai'i. However, the number of populations sampled was small (concern about the status of *L. concolor* precluded an extensive survey). Neither investigation described the pattern of gene flow among populations and islands, nor assessed the probable proportion of migrants in individual populations. This study was undertaken to further describe the population genetics of *N. granosa* and to use this information to assess the spatial pattern and degree of larval dispersal among stream populations occurring throughout the Hawaiian Archipelago.

Locally known as *hīhīwai, N. granosa* is the largest of the Hawaiian freshwater neritids (Maciolek 1978). It is a coveted subsistence food item for Native Hawaiians. Most of its basic biology is known from Ford (1979). The largest individuals reach 50 mm in shell length. Sexual maturity occurs at about 12 mm shell length. The species exhibits separate sexes and iteroparity. Individuals may live from 1 to 7 yr. Sessile egg capsules are affixed directly to the substrate. After hatching, larvae are swept to the sea. The shells of the veliger larvae are 150–175 μm in diameter at hatching and 540–660 μm at settlement. Both reproduction and recruitment occur year-round but with some periodicity (Ford 1979, Hodges 1992; M.H.H., unpubl. obs.; C. Way, pers. comm.). Based on growth studies in the laboratory, Ford (1979) estimated the length of time spent by the larvae in the marine environment at close to a year. However, the actual length of time is unknown and seems likely to be less than that. Sometime after returning to a stream from the ocean, individuals ranging from about 5 to 15 mm shell length spread throughout the adult habitat by migrating upstream in long trains (Ford 1979, Hodges 1992).

MATERIALS AND METHODS

M.H.H. collected samples of *N. granosa* from three streams on each of four islands (Figure 1). These are Hanakāpī'ai (159° 36′ W, 22° 12′ N), Wainiha (159° 32′ W, 22° 12′ N), and Hanalei (159° 30′ W, 22° 13′ N) on Kaua'i; Waioho'okalo (156° 54′ W, 21° 10′ N), Wailau (156° 50′ W, 21° 10′ N), and Kawainui (156° 48′ W, 21° 10′ N) on Moloka'i; Honomanū (156° 10′ W, 20° 52′ N), Waiohue (156° 07′ W, 20° 49′ N), and Hanawī (156° 06′ W, 20° 49′ N) on Maui; and Nānue (155° 09′ W, 19° 55′ N), Hakalau (155° 07′ W,

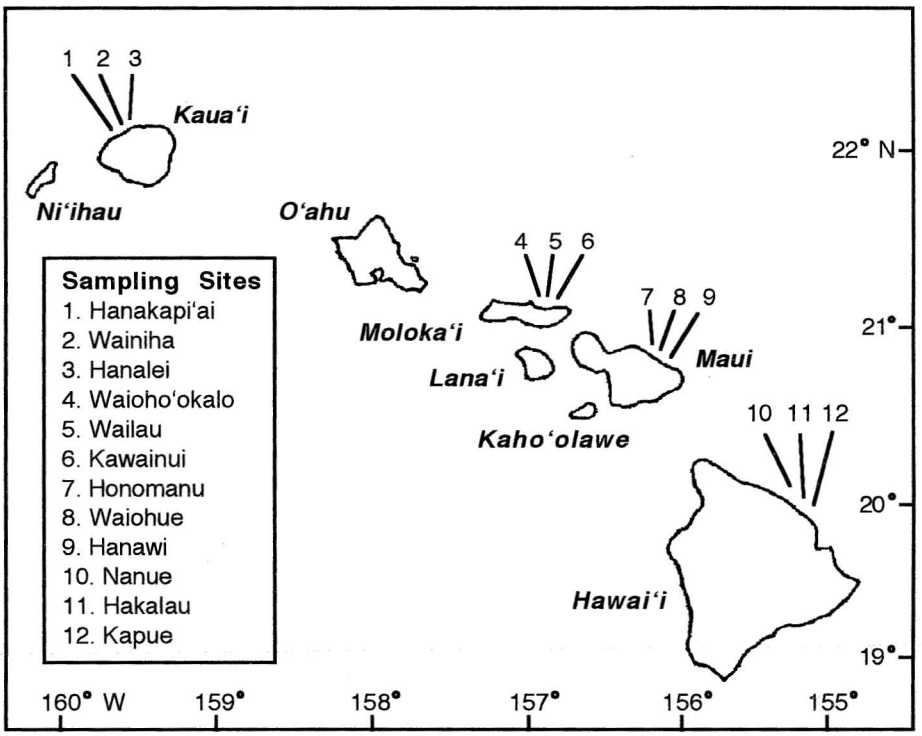

FIGURE 1. Location of sample streams.

19° 54′ N), and Kapue (155° 05′ W, 19° 47′ N) on Hawai'i. Each stream was sampled over the span of a few hours. Sampling occurred between July and September 1991.

Samples were collected at the terminus of each stream except Hanalei, where samples were collected where the first riffle occurs approximately 5.5 km inland and above the range of tidal influence. Straight-line distances among the termini of sample streams range from 2 to 540 km.

To examine within-stream heterogeneity, samples were collected approximately 1500 m and 700 m upstream of the termini in Hanakāpī'ai and Hanawī Streams, respectively, for comparison with samples taken at the termini of these two streams. The samples collected at all locations exhibited the "lower stream" shell morph of Ford (1979).

Collection of samples in streams was restricted to as small an area as possible (usually ca. 70 m^2). Only adults (greater than 12 mm shell length) were collected. Samples were transported alive to freezers, then brought to the laboratory for analysis.

Protein Electrophoresis

We used both foot and hepatopancreas tissue for assay at each presumptive locus. Tissues were homogenized in 10 ml of 0.25 g/liter pyridoxal-5-phosphate grinding solution and centrifuged at 5000 rpm for 5 min. Gels were made with 12% hydrolyzed Sigma starch. Gel buffer systems used were AC (gel buffer: 0.002 M citric acid, pH adjusted with N-(3-aminopropyl)-morpholine and citric acid; electrode buffer: 0.04 M citric acid, pH 6.1 [Clayton and Tretiak 1972]); MF (stock solution: 0.9 M tris, 0.5 M boric acid, 0.02 M EDTA, pH 8.7; gel buffer: 1:20 dilution of stock solution; electrode buffer: 1:5 dilution

TABLE 1

ENZYMES USED IN ELECTROPHORETIC SURVEY OF *N. granosa* IN HAWAI'I

ENZYME[a]	E.C.	BUFFER SYSTEM[b]
Aspartate aminotransferase (AAT)	2.6.1.1	AC (pH 6.1)
Acid phosphatase (ACP-1)	3.1.3.2	AC (pH 6.1)
(ACP-2)	"	"
Glyceraldehyde-3-phosphate dehydrogenase (G3PDH)	1.2.1.12	AC (pH 6.9)
Glucose-6-phosphate isomerase (GPI)	5.3.1.9	RW
Isocitrate dehydrogenase (IDHP-1)	1.1.1.42	AC (pH 6.9)
(IDHP-2)	"	"
Leucine aminopeptidase (LAP)	3.4.11.1	RW
Malate dehydrogenase (MDH)	1.1.1.37	AC (pH 6.9)
Mannose-6-phosphate isomerase (MPI)	5.3.1.8	RW
Phenylalanyl-proline (PEP)	3.4.1.1	MF
6-phosphogluconate dehydrogenase (PGDH)	1.1.1.44	AC (pH 6.9)
Phosphoglucomutase (PGM)	5.4.2.2	RW
Sorbitol dehydrogenase (SDH)	1.1.1.14	RW
Superoxide dismutase (SOD)	1.15.1.1	RW

[a] Nomenclature from Shaklee et al. (1990).
[b] See text for buffer descriptions.

of stock solution [Market and Faulhaber 1965]); RW (gel buffer: 0.03 M tris, 0.005 M citric acid, pH 8.5; electrode buffer: 0.06 M lithium hydroxide, 0.03 M boric acid, pH 8.1 [Ridgway et al. 1970]). We followed the electrophoretic methods of Leary and Booke (1990) and the histochemical staining techniques of Harris and Hopkinson (1976).

We assembled a list of 50 enzymes from the literature that had been successfully screened in other gastropods. Fifteen of those enzymes stained reliably, and we scored these for approximately 30 individuals per stream population. Enzymes and buffer systems are given in Table 1. Isozymes of multilocus systems are numbered from cathode to anode. Alleles are numbered according to relative electrophoretic mobility and presented according to approximate frequency of occurrence: *AAT*100, *110, *80, *90; ACP-1*-100, *-180, *-60; GPI*100, *225, *25; IDHP-2*100, *5, *200, *120; MPI*100, *115, *60; PEP*100, *109, *97; PGDH*100, *275, *-50; PGM*100, *108, *90, *95, *80; SDH*100, *86, *106.*

We used the BIOSYS-1 computer program (Swofford and Selander 1989) to analyze electromorph frequencies. Levene's (1949) correction for small sample size was applied in tests for deviation from Hardy-Weinberg proportions.

We quantified heterozygote deficiency with $D^* = (H_o - H_e)/H_e$, where H_o is observed heterozygosity and H_e is expected heterozygosity. This is simply Wright's fixation index with a negative sign, but emphasizes deficiency (Zouros et al. 1980). Homogeneity of heterozygote deficiencies was tested by treating D^* as a correlation coefficient (Sokal and Rolf 1981, Gaffney et al. 1990).

RESULTS

Of the 15 loci assayed in *N. granosa,* nine were polymorphic and the overall mean heterozygosity was 0.052 (Table 2). Eight of the 12 populations deviated significantly from Hardy-Weinberg proportions at some loci (Table 2). These deviations occurred at six of nine polymorphic loci. Deviations were observed in 17 out of 83 tests, more than expected by chance alone at $P < .05$. All significant deviations were as deficiencies of heterozygotes. The mean value of D^* was -0.122.

There was no detectable difference in fre-

TABLE 2

ALLELE FREQUENCIES AT NINE LOCI IN 12 POPULATIONS OF *N. granosa* IN HAWAI'I
(H_o IS OBSERVED HETEROZYGOSITY, H_e IS THE HETEROZYGOSITY EXPECTED UNDER HARDY-WEINBERG EQUILIBRIUM, AND D^* IS HETEROZYGOTE DEFICIENCY)

LOCUS	POPULATION	*n*	ALLELE FREQUENCY					H_o	H_e	D^*
AAT			**100*	**110*	**80*	**90*				
	Hanakāpī'ai	30	0.733	0.233	0.033	—	—	0.133	0.407	−0.672***
	Wainiha	28	0.679	0.304	0.018	—	—	0.286	0.447	−0.361
	Hanalei	30	0.550	0.450	—	—	—	0.433	0.495	−0.125
	Waioho'okalo	30	0.767	0.217	—	0.017	—	0.300	0.365	−0.178
	Wailau	30	0.733	0.217	0.033	0.017	—	0.267	0.414	−0.356
	Kawainui	24	0.458	0.458	0.042	0.042	—	0.625	0.576	0.084
	Honomanū	30	0.900	0.050	0.050	—	—	0.200	0.185	0.081
	Waiohue	30	0.700	0.283	—	0.017	—	0.267	0.429	−0.379*
	Hanawī	30	0.667	0.317	0.017	—	—	0.267	0.455	−0.414*
	Nānue	28	0.672	0.293	0.034	—	—	0.379	0.461	−0.177
	Hakalau	24	0.688	0.250	0.042	0.021	—	0.292	0.463	−0.370
	Kapue	30	0.667	0.333	—	—	—	0.333	0.444	−0.250
										mean = −0.260
ACP-1			**-100*	**-180*	**-60*					
	Hanakāpī'ai	29	0.983	0.017	—	—	—	0.034	0.034	0.018
	Wainiha	29	0.948	0.034	0.017	—	—	0.103	0.099	0.042
	Hanalei	29	0.966	0.034	—	—	—	—	0.067	−1.00***
	Waioho'okalo	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Wailau	30	1.00	—	—	—	—	—	—	—
	Kawainui	26	0.885	0.096	0.019	—	—	0.231	0.208	0.110
	Honomanū	30	1.00	—	—	—	—	—	—	—
	Waiohue	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Hanawī	30	0.950	0.050	—	—	—	0.100	0.095	0.053
	Nānue	30	1.00	—	—	—	—	—	—	—
	Hakalau	30	1.00	—	—	—	—	—	—	—
	Kapue	30	1.00	—	—	—	—	—	—	—
										mean = −0.106
GPI			**100*	**225*	**25*					
	Hanakāpī'ai	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Wainiha	30	1.00	—	—	—	—	—	—	—
	Hanalei	30	0.967	—	0.033	—	—	—	0.064	−1.00***
	Waioho'okalo	30	0.967	0.017	0.017	—	—	0.067	0.065	0.026
	Wailau	30	0.950	0.050	—	—	—	0.100	0.095	0.053
	Kawainui	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Honomanū	30	0.983	—	0.017	—	—	0.033	0.033	0.017
	Waiohue	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Hanawī	30	0.967	0.033	—	—	—	0.067	0.064	0.034
	Nānue	30	0.917	0.067	0.017	—	—	0.167	0.155	0.075
	Hakalau	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Kapue	30	0.967	0.017	0.017	—	—	0.067	0.065	0.026
										mean = −0.064
IDHP-2			**100*	**5*	**200*	**120*				
	Hanakāpī'ai	30	0.950	0.017	0.017	0.017	—	0.100	0.097	0.034
	Wainiha	29	0.983	—	0.017	—	—	0.034	0.034	0.018
	Hanalei	27	0.963	—	0.037	—	—	0.074	0.071	0.038
	Waioho'okalo	30	1.00	—	—	—	—	—	—	—
	Wailau	30	1.00	—	—	—	—	—	—	—
	Kawainui	30	0.967	0.017	—	0.017	—	0.067	0.065	0.026
	Honomanū	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Waiohue	30	1.00	—	—	—	—	—	—	—

TABLE 2 (continued)

LOCUS	POPULATION	n	ALLELE FREQUENCY					H_o	H_e	D^*
	Hanawī	29	0.966	0.034	—	—	—	0.069	0.067	0.036
	Nānue	30	1.00	—	—	—	—	—	—	—
	Hakalau	30	0.967	0.017	0.017	—	—	0.067	0.065	0.026
	Kapue	30	0.983	—	0.017	—	—	0.033	0.033	0.017
										mean = 0.027
MPI			*100	*115	*60					
	Hanakāpīʻai	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Wainiha	29	0.931	0.052	0.017	—	—	0.069	0.130	−0.470*
	Hanalei	27	0.889	0.111	—	—	—	0.074	0.198	−0.625**
	Waiohoʻokalo	28	0.857	0.071	0.071	—	—	0.214	0.255	−0.160
	Wailau	26	0.904	0.058	0.038	—	—	0.077	0.178	−0.568***
	Kawainui	29	0.929	0.036	0.036	—	—	0.071	0.135	−0.472
	Honomanū	30	0.967	—	0.033	—	—	0.067	0.064	0.034
	Waiohue	29	0.914	0.034	0.052	—	—	0.172	0.161	0.070
	Hanawī	30	0.933	0.050	0.017	—	—	0.067	0.126	−0.471*
	Nānue	30	0.950	0.050	—	—	—	0.100	0.095	0.053
	Hakalau	29	0.948	0.017	0.034	—	—	0.103	0.099	0.042
	Kapue	30	0.850	0.100	0.050	—	—	0.233	0.265	−0.119
										mean = −0.222
PEP			*100	*109	*97					
	Hanakāpīʻai	30	0.867	0.083	0.050	—	—	0.267	0.239	0.114
	Wainiha	29	0.948	0.034	0.017	—	—	0.034	0.099	−0.653***
	Hanalei	27	0.950	0.050	—	—	—	0.100	0.095	0.053
	Waiohoʻokalo	28	0.983	—	0.017	—	—	0.033	0.033	0.017
	Wailau	26	0.875	0.125	—	—	—	0.107	0.219	−0.510**
	Kawainui	29	0.900	0.100	—	—	—	0.067	0.180	−0.630**
	Honomanū	30	0.950	0.017	0.033	—	—	0.100	0.096	0.040
	Waiohue	29	0.950	0.017	0.033	—	—	0.100	0.096	0.040
	Hanawī	30	0.942	0.038	0.019	—	—	0.115	0.110	0.047
	Nānue	30	0.950	0.050	—	—	—	0.100	0.095	0.053
	Hakalau	29	0.883	0.083	0.033	—	—	0.233	0.212	0.102
	Kapue	30	0.933	0.033	0.033	—	—	0.067	0.127	−0.474*
										mean = −0.150
PGDH			*100	*275	*-50					
	Hanakāpīʻai	27	1.00	—	—	—	—	—	—	—
	Wainiha	30	1.00	—	—	—	—	—	—	—
	Hanalei	27	1.00	—	—	—	—	—	—	—
	Waiohoʻokalo	30	0.967	0.017	0.017	—	—	0.067	0.065	0.026
	Wailau	30	0.983	—	0.017	—	—	0.033	0.033	0.017
	Kawainui	30	0.950	0.033	0.017	—	—	0.033	0.096	−0.653***
	Honomanū	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Waiohue	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Hanawī	30	0.933	0.067	—	—	—	0.067	0.124	−0.464*
	Nānue	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Hakalau	30	1.00	—	—	—	—	—	—	—
	Kapue	30	0.933	0.017	0.050	—	—	0.067	0.126	−0.471*
										mean = −0.187
PGM			*100	*108	*90	*95	*80			
	Hanakāpīʻai	30	0.967	0.017	—	0.017	—	0.067	0.065	0.026
	Wainiha	30	0.967	—	0.033	—	—	0.067	0.064	0.034
	Hanalei	30	0.950	0.017	—	0.017	0.017	0.100	0.097	0.034
	Waiohoʻokalo	30	1.00	—	—	—	—	—	—	—

TABLE 2 (continued)

LOCUS	POPULATION	*n*	ALLELE FREQUENCY					H_o	H_e	D^*
	Wailau	30	0.950	0.017	0.017	0.017	—	0.100	0.097	0.034
	Kawainui	30	0.983	—	—	—	0.017	0.033	0.033	0.017
	Honomanū	30	0.983	—	—	0.017	—	0.033	0.033	0.017
	Waiohue	30	0.917	0.067	—	—	0.017	0.167	0.155	0.075
	Hanawī	30	0.983	0.017	—	—	—	0.033	0.033	0.017
	Nānue	30	0.967	0.033	—	—	—	0.067	0.064	0.034
	Hakalau	30	0.983	—	0.017	—	—	0.033	0.033	0.017
	Kapue	30	0.967	0.017	0.017	—	—	0.067	0.065	0.026
										mean = 0.030
SDH			**100*	**86*	**106*					
	Hanakāpīʻai	23	1.00	—	—	—	—	—	—	—
	Wainiha	24	0.958	0.021	0.021	—	—	0.083	0.081	0.032
	Hanalei	30	1.00	—	—	—	—	—	—	—
	Waiohoʻokalo	30	1.00	—	—	—	—	—	—	—
	Wailau	29	0.983	—	0.017	—	—	0.034	0.034	0.018
	Kawainui	30	1.00	—	—	—	—	—	—	—
	Honomanū	30	1.00	—	—	—	—	—	—	—
	Waiohue	29	1.00	—	—	—	—	—	—	—
	Hanawī	30	1.00	—	—	—	—	—	—	—
	Nānue	29	1.00	—	—	—	—	—	—	—
	Hakalau	30	1.00	—	—	—	—	—	—	—
	Kapue	30	1.00	—	—	—	—	—	—	—
										mean = 0.025

Chi-square test for deviation from Hardy-Weinberg expectations: *, $P < .05$; **, $P < .01$; ***, $P < .001$.

TABLE 3

SUMMARY OF *F*-STATISTICS FOR 12 POPULATIONS OF *N. granosa* IN HAWAIʻI

LOCUS	NO. OF ALLELES	F(IS)	F(ST)	F(IT)
AAT	4	0.264	0.049*	0.300
ACP-1	3	0.058	0.038*	0.094
GPI	3	0.059	0.016	0.074
IDHP-2	4	−0.029	0.015	−0.014
MPI	3	0.263	0.020	0.278
PEP	3	0.173	0.021	0.190
PGDH	3	0.324	0.024*	0.341
PGM	5	−0.038	0.016	−0.022
SDH	3	−0.028	0.023	−0.004
Mean		0.198	0.034*	0.225

* *Fst* significantly >0: chi-square test (Workman and Niswander 1970).

quencies between upstream and downstream samples (Hanakāpīʻai, df = 18, $P = .248$, $\chi^2 = 21.6$; Hanawī, df = 14, $P = .619$, $\chi^2 = 11.8$). This suggests genetic homogeneity within streams among individuals of the lower shell morph.

Wright's (1951) *F*-statistics, which provide an index of the degree of genetic subdivision, are given in Table 3. *Fst* at *AAT**, *ACP-1**, and *PGDH** is significantly greater than zero ($P < .05$, chi-square test [Workman and Niswander 1970]). Average *Fst* (0.034) was also significant. This indicates significant genetic heterogeneity among the 12 stream populations. Pairwise chi-square tests among all populations resulted in 12 differences out of 66 comparisons, more than expected by chance at $P < .05$. All differences involved Hanalei (Kauaʻi), Kawainui (Molokaʻi), and Honomanū (Maui). Thus the significance of overall *Fst* is largely due to differences among the samples from Hanalei, Kawainui, and Honomanū Streams, and between these and the remaining populations.

The allele frequencies that cause heterogeneity are clear at *AAT** and *ACP-1**. The

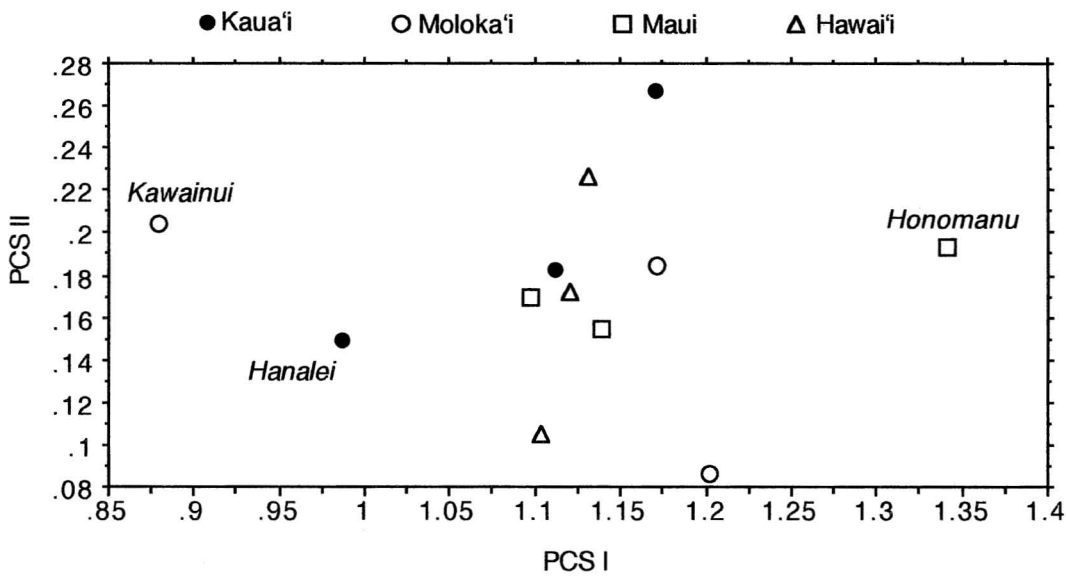

FIGURE 2. Plot of first two principal component scores of allele frequencies at nine polymorphic loci in 12 populations. Different symbols denote islands on which populations occur. The three populations that are genetically distinct are labeled.

allele indicated by *AAT*110* is at high frequency in Hanalei and Kawainui and is rare in Honomanū (Table 2). That by *ACP*-180* is at comparatively high frequency in Kawainui. Such notable differences are not apparent at *PGDH**.

A principal components analysis performed on the covariance of the frequency of the common alleles indicated a lack of geographic structure. The first principal component, which accounts for 69.8% of the variance in allele frequencies, is plotted against the second principal component, which accounts for an additional 13.4% (Figure 2). The first component (PC 1) is based primarily on differences at *AAT** and *ACP-1**. The second (PC 2) is based on differences at *MPI** and *PEP**. The first component separates Kawainui and Honomanū, and to a lesser extent Hanalei from the central cluster. Despite this, the genetic structure is consistent with an island model of migration.

DISCUSSION

Genetic Variation within Populations

The heterozygote deficiency observed in *N. granosa* is common in mollusks (Volckaert and Zouros 1989) and marine invertebrates (Grant and Lang 1991). Gartner-Kepkay et al. (1980, 1983) reported a mean D^* of -0.492 and -0.396 in the mussel *Mytilus edulis*. Data from Zouros et al. (1980) gave a mean D^* of -0.298 for oysters (*Crassostrea virginica*). The scallop *Placopecten magellanicus* exhibited much lower D^* (-0.065 [Volckaert and Zouros 1989]). At -0.122, mean D^* in *N. granosa* was intermediate in relation to those other species.

Heterozygote deficiency in marine invertebrates is a contentious issue. Hypotheses most often presented are the Wahlund effect (genetic subdivision within the sample), null alleles (alleles that fail to appear in the electrophoresis process), nonrandom mating, and natural selection (Gartner-Kepkay et al. 1980, 1983, Zouros et al. 1980, 1988, Mallet et al.

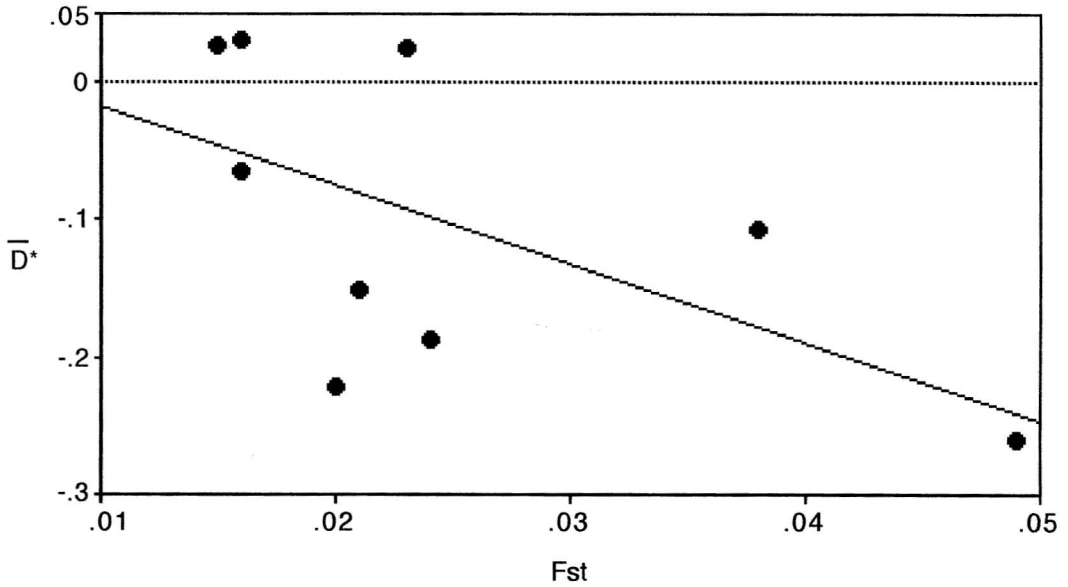

FIGURE 3. Mean heterozygote deficiency plotted against *Fst* at each locus (simple regression, one-tailed test: $r^2 = 0.345$, $P < .05$).

1985, Foltz 1986, Volckaert and Zouros 1989, Gaffney et al. 1990).

Though stream habitats are discrete, genetically distinct cohorts might occur within the same stream (e.g., Johnson and Black 1984, Mallet et al. 1985), thus causing genetic subdivision within the stream sample and concomittant heterozygote deficiency (the Wahlund effect). If a Wahlund effect is caused by distinct larval cohorts within samples, deficiency should be stronger at those loci that show more differentiation among populations (Volckaert and Zouros 1989). A plot of D^* for each locus averaged among populations against *Fst* at that locus suggests a negative trend (Figure 3). This supports the possibility of a Wahlund effect. Note however that this trend is weak and is heavily influenced by the single data point corresponding to *AAT**.

If null homozygotes are lethal or are excluded from the sample the expected frequency of null alleles in *N. granosa* given mean D^* is 0.334 (Gaffney et al. 1990). If nulls are selectively neutral and only one null allele segregates per locus, the frequency necessary to account for the observed deficiency is of the same magnitude. These estimates are orders of magnitude larger than frequencies determined for *Drosophila* (Langley et al. 1981) and pine trees (Allendorf et al. 1982), but similar to frequencies inferred by Foltz (1986) for an oyster. However, it is unlikely that null alleles will be segregating at such high frequency at six of nine loci.

Nonrandom mating will cause uniform deficiency among loci (Lewontin and Krakauer 1973, Zouros et al. 1980). D^* is heterogeneous at five of the 12 populations (Hanakāpīʻai, Wainiha, Hanalei, Wailau, Kawainui; tested as correlation coefficients, $P < .05$; see *Materials and Methods*). In addition, although adults are very sedentary and fertilization is internal, the observation of within-stream genetic homogeneity of the shell morph used in this assay, coupled with the fact that recruits migrate throughout the adult habitat, suggest that nonrandom mating is an unlikely cause of the deficiency.

Selection is difficult to document, especially in retrospect. Mallet et al. (1985) found evidence for selective larval mortality in mussels, but noted that the Wahlund effect (e.g., Burton 1983) and inbreeding were most often invoked as causes of deficiency. Both Mallet et al. (1985) and Zouros et al. (1988) stated that there is no consensus on the cause of heterozygote deficiencies. Similarly, it is not clear what causes deficiency in *N. granosa.*

Among-Population Heterogeneity

Heterogeneity in a high gene flow system suggests selection. Gartner-Kepkay et al. (1980, 1983) observed heterogeneity in mussels despite the high gene flow expected of planktonic larvae and suggested selective environmental effects. Campton et al. (1992) documented temporal and spatial genetic patchiness in the conch *Strombus gigas.* Heterogeneity exists on temporal and microgeographic scales in the limpet *Siphonaria jeanae* (Johnson and Black 1984). Those authors concluded that differentiation was caused by unique histories of localized recruitment that may have been driven by selective processes operating on the larvae.

Either stream-specific selection acting on postsettlement individuals within the stream habitat or selection acting on larvae at sea and thereby producing genetically distinct recruitment events could cause the heterogeneity observed in *N. granosa.* If stream-specific selection is the cause, the pattern of heterogeneity will remain constant through time. On the other hand, if at-sea selection is the cause, the locations of genetically distinct populations are likely to change. Time-series allele frequency data are needed to investigate the relative importance of stream-specific selection.

Genetic subdivision within populations can cause heterogeneity between populations. Such subdivision would likely manifest itself as heterozygote deficiency within populations (i.e., the Wahlund effect). Heterozygote deficiencies do occur in the samples, and the Wahlund effect has not been eliminated as a cause of such deficiency. However, there was no detectable difference among populations in the number of loci that exhibited significant heterozygote deficiencies (chi-square = 8.715, df = 11, $P = .65$; note that sparse cells require extreme caution in interpreting this test). And, although significant deficiencies occur at Hanalei and Kawainui, D^* is positive for all loci in Honomanū. Thus, it appears that any genetic subdivision occurring within the populations is probably not a cause of the observed heterogeneity among the populations.

Neutral processes can cause significant heterogeneity among populations under conditions of considerably higher gene flow than that calculated for *N. granosa* (Allendorf and Phelps 1981). If drift is the cause of heterogeneity, one would expect to see equal divergence among polymorphic loci. However, although the values of *Fst* are similar among the nine ploymorphic loci, *Fst* is significant at three of them (Table 3).

In sum, there are a number of processes that could contribute to the genetic heterogeneity observed among the populations of *N. granosa.* These include stream-specific selection acting on postsettlement individuals, at-sea selection acting on larvae and producing genetically distinct recruitment events, and neutral processes. However, the relative importance of each cannot be determined with existing information.

The genetic structure (Figure 2) is consistent with an island model of migration. Thus migrants originate from any stream throughout the Islands with equal probability.

Amount of Dispersal

A simple estimate of dispersal among populations can be made using Wright's (1969) equation:

$$Fst = 1/(4N_em + 1) \qquad (1)$$

and solving for N_em (Waples 1987, Slatkin and Barton 1989), where N_e is the genetically effective subpopulation size and m is the proportion of the subpopulation that is made up of migrants. Dispersal is in terms of the absolute number of migrants per generation N_em (gene flow) and is independent of subpopulation size (Allendorf and Phelps 1981).

Equation 1 assumes selective neutrality and an island model of migration. Thus, determination of N_em from *Fst* rests on the assumption of selective neutrality. However, N_em is fairly robust to selection where the level of gene flow is on the order of that determined here (Slatkin and Barton 1989). Likewise, Figure 2 demonstrates that the pattern of differences in allele frequencies is consistent with the island model. Overall *Fst* from Table 3 is 0.034. Equation 1 yields 7.103 for N_em. Approximately seven migrants are moving among streams each generation.

Genetic Versus Demographic Importance of Migration

From a genetic point of view the N_em (= 7) calculated for *N. granosa* is high and indicates sufficient gene flow to maintain similar allele frequencies (Hartl and Clark 1989:318). A little migration goes a long way toward affecting genetic processes, and the amount of migration necessary to maintain genetic homogeneity is independent of subpopulation size (Allendorf and Phelps 1981). However, where populations are large, a correspondingly large amount of migration is required to control, or even affect, demographic dynamics.

Hodges (1992) reported populations of *N. granosa* ranging from 70,000 to 350,000 individuals in three streams on Maui. Many other stream populations are probably of a similar size, and some may well be larger. The rate of migration (seven individuals per generation) inferred from these genetic data might not be sufficient to affect demographic processes in such large populations of *N. granosa.*

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