

Biofouling likely serves as a major mode of dispersal for the polychaete tubeworm *Hydroides elegans* as inferred from microsatellite loci

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Abstract

The polychaete tubeworm *Hydroides elegans* (Haswell) is a biofouling species with relatively limited larval dispersal. Four highly polymorphic microsatellite loci were used to make inferences about the migration and global population structure of 137 individuals from seven sub-populations located in the Atlantic, Pacific, and Indian Oceans and in the Mediterranean Sea. The results of the genetic analyses suggest minimal population sub-structure ($F_{st} = 0.09$). Estimates of pairwise F_{st} and migration rates using the coalescent-based method of MIGRATE suggest that there is little genetic differentiation between certain populations. Variation in relatedness among pairs of populations is consistent with a suite of local and global factors. The most likely explanation for close genetic relatedness among certain populations over such vast distances is the regular and consistent transport of adults and larvae on the hulls and in the ballast water of ships, respectively.

Introduction

For benthic marine invertebrates with a sessile adult phase and a mobile larval phase, larvae are often an important factor influencing the amount of migration between adult populations (e.g. Thorson, 1950; Olson, 1985; Strathmann, 1985; Wares et al. 2001). Larval dispersal is affected by factors such as the duration of the larval swimming phase (e.g. Wendt, 1996; 1998), larval behavior (Forward et al. 1984; Young, 1995), and the inherent physiological plasticity of larvae to withstand environmental variability, i.e. changes in water temperature across oceanographic boundaries (Scheltema, 1986; Wares et al. 2001). Abiotic factors such as ocean currents may also influence larval dispersal. The potential for adults to travel great distances by rafting on natural objects and on artificial objects such as ships' hulls (i.e. biofouling) or in ballast water, represent additional mechanisms of migration (Ruiz et al. 2000).

Thorson (1950) described the relationship between larval lifespan and dispersal as one in which those species having long-lived larvae disperse greater distances than those with short-lived larvae. The expected dispersal potential of larvae, often inferred

from larval lifespan, has subsequently been used to predict the relative degree of gene flow among sub-populations. It is often assumed that, over similar distances, species with short-lived larvae will show greater genetic differentiation between populations than those with long-lived larvae (Palumbi, 1994; 1995). The persistence of this general paradigm may in large part be due to the difficulty of monitoring larvae across great distances (Palumbi, 1994; 1995) and the relative ease of inferring dispersal potential from laboratory experiments on larval lifespan (Lambert et al. 2000). Direct studies of larval dispersal potential in natural habitats are rare and have been performed exclusively on species that have relatively large, short-lived larvae (e.g. Olson, 1985). Previous research on marine invertebrates employing genetic markers to investigate dispersal potential has both supported and contradicted the paradigm relating the longevity of the larval phase and the amount of gene flow between distant populations. For example, Solé-cava et al. (1994) found high genetic similarity at 13 loci coding for 11 enzymes between distant subpopulations of the sea anemone, *Utricina eques*, which is believed to have low dispersal

potential. In contrast, McMillan et al. (1992) analysed mitochondrial DNA from the sea urchin *Heliocidaris* sp. that also has short-lived larvae, and found evidence that supported the *a priori* hypothesis of significant population structure.

The fouling characteristics of polychaete tube-worms, which represent an additional mechanism of dispersal, are well documented. The serpulid polychaete tubeworm *Hydroides elegans*, the focal species of this study, is considered to be the primary fouler in the Mediterranean Sea (Koçak et al. 1999) and the waters surrounding Australia (Lewis et al. 2006). Other species in the genus (e.g. *H. dianthus*, *H. sanctaerucii*, *H. ezoensis*) are also common foulers (Çinar et al. 2006; Lewis et al. 2006). Historically the interest in the fouling characteristics of these polychaetes and other organisms was linked to their effects on fuel consumption due to an increase in drag on ships, the damage done to the surface of ships and marine piping networks, all of which increase economic costs. More recently, an increased understanding of the negative effects of introduced species on native communities has presented an additional concern for the transport of biofouling species (e.g. Wasson et al. 2002). Indeed, *H. elegans* and *H. dianthus* were among seven exotic species found in Izmir Bay, Aegean Sea (Çinar et al. 2006), and *H. sanctaerucis*, which is native to the Caribbean, has recently been documented for the first time in Northern Australia (Lewis et al. 2006). In both studies, hull fouling was believed to be the primary mechanism of introduction and the potential negative effects on native marine community structure were cited as concerns.

The cosmopolitan *H. elegans*, which has a sessile adult stage and a motile larval stage, is a gonochoristic serpulid polychaete tubeworm with a global geographic distribution restricted to tropical and sub-tropical coastal marine environments (Walters et al. 1997). *H. elegans* is a broadcast spawner whereby females and males release gametes into the water column where fertilisation occurs (Hadfield, 1998). Under laboratory conditions, fertilisation occurs within 24 h, and embryos develop into trochophore larvae (Hadfield, 1998), which become competent to metamorphose 4–6 days after fertilisation (Hadfield et al. 1994; Walters et al. 1997; Carpizo-Ituarte & Hadfield, 1998; Holm et al. 1998; Qian & Pechenik, 1998). Once competent, larvae, which have a swimming phase of at least 4 weeks, respond to a variety of natural environmental cues (Hadfield et al. 1994; Carpizo-Ituarte & Hadfield, 1998; Beckmann et al. 1999) and specific bacterial biofilms (Unabia & Hadfield, 1999; Lau & Qian, 2001; Huang & Hadfield, 2003; Dobretsov et al. 2006). The onset of metamorphosis is also triggered by artificial cues (Bryan et al. 1997; Qian & Pechenik, 1998) and

naturally occurring bioorganic films (e.g. those found on ships' hulls) (Hadfield et al. 1994; Walters et al. 1997). The sensitivity and receptiveness of larvae to these cues varies depending on concentration, the temperature of the surrounding medium (Qian & Pechenik, 1998), and the age of the larvae (Carpizo-Ituarte & Hadfield, 1998).

Given the relatively short duration of larval swimming (*ca* 4 weeks) for *H. elegans*, if natural means are the sole mode of dispersal, then significant genetic heterogeneity across large distances such as ocean basins should be observed. If, in contrast, biofouling on ships' hulls or larval transport in ballast water were occurring regularly, relative panmixia across vast oceanographic distances should be observed. In this study, four polymorphic micro-satellite loci were analysed to infer levels and patterns of genetic structure in *H. elegans* populations. In addition to estimating the degree of population structure through *F*-statistics, recent methods based on coalescent theory for estimating migration were also employed. These coalescent-based methods are able to estimate asymmetric migration rates (Beerli & Felsenstein, 1999) and avoid many of the problems associated with deriving migration rates from *F_{st}* values (Whitlock & McCauley, 1999). Given that *H. elegans* is a dominant fouler in marine ecosystems and of ships' hulls, it was expected that the analysis of molecular markers would demonstrate that despite the relatively limited larval dispersal of *H. elegans*, there is a high degree of gene flow and little genetic differentiation between distant populations.

Methods

Sample collection

Specimens of *Hydroides elegans* (Haswell) were acquired from seven locations in three ocean basins including the Atlantic Ocean (St Petersburg and Sarasota Florida, USA), the Pacific Ocean (Pearl Harbor Hawaii, USA; Sydney Harbor, Sydney, Australia; N. Taiwan and S. Taiwan, Taiwan), and the confluence of the Pacific and Indian Oceans (Singapore Harbor, Singapore). Individuals from the Mediterranean Sea (Lake Tunisia, Tunisia) (Table I) were also acquired. Specimens were fixed in 70–90% ethanol and stored at 4°C. All of the worms were collected from 1999–2002 (Table I). The taxonomic identity of all individuals was assessed based on characteristics of the operculum, which is a key characteristic in polychaete taxonomy. Ideal sample sizes were difficult to obtain and the difficulty in isolating stable high molecular weight DNA also restricted the strength of some analyses.

Table I. The location, collection date, and number of individuals (n) of the seven populations of *H. elegans*.

Population names	Location	Ocean basin	Coordinates	Collection date	n
Hawaii	Pearl Harbor Research Station, Hawaii	Central Pacific	21°16' N 157°42' W	Nov. 2001	23
Australia	Sydney harbor and Port Jackson, Australia	Pacific	33°55' S 151°17' E	Jun. 1999	25
Florida	Mote Marine Lab Sarasota, Florida	Gulf of Mexico	27°20' N 82°35' W	Feb 2002	47
	St Petersburg, Florida		27°45' N 82°38' W	Dec. 2001	
Singapore	Pulau Ubin, Singapore	S. China Sea/Indian Ocean	1°22' N 103°45' E	May 2002	9
Tunisia	Lake Tunis, Tunisia	Mediterranean Sea	36°48' N 10°16' E	May 2002	12
N. Taiwan	Keelung Harbor, Taiwan	Pacific/Philippine Sea	25°02' N 121°38' E	June 2002	9
S. Taiwan	Kaohsiung Harbor, Taiwan	Pacific/Philippine Sea	22°30' N 121°02' E	April 2002	12

Sequencing and genotyping

The DNA extraction protocol and development of the microsatellite library used were fully described by Pettengill et al. (2003). Genotyping reactions for population structure analyses were performed in a 10 μ l volume containing 5.175 μ l H₂O, 1.8 μ l 10X buffer, 0.8 μ l 25 mM MgCl₂, 0.4 μ l 10 mM dNTPs, 0.125 μ l of Promega Taq DNA polymerase, 0.5 μ l 1 pmol IRD 700 or IRD 800 (Licor) labeled forward and reverse primers. The reaction products were loaded on a 25 cm gel (8.4 g urea, 4 ml 5X TBE, 2.4 ml LongRanger acrylamide, 200 μ l APS, and 15 μ l TEMED) with 0.25 mm spacers. Scoring of genotypes was conducted using LiCor's Gene ImagIR[®] software.

Analysis of microsatellite genotypes

The observed and expected heterozygosity, the number of unique alleles per population, the range of allele sizes, and the mean number of alleles per locus were quantified using a Microsatellite Analyser (MSA) (Dieringer & Schlotterer, 2003). The frequencies of null alleles (r) were calculated as $(H_{\text{exp}} - H_{\text{obs}})/(1 + H_{\text{e}})$ (Brookfield, 1996). Additionally, a test for strong effects of null alleles was conducted using Micro-Checker (van Oosterhout et al. 2004). In those instances where the presence of null alleles was sufficient, an adjusted genotype file, which included an adjustment accounting for the presence of null alleles (Chakraborty et al. 1992), was obtained from Micro-Checker (van Oosterhout et al. 2004). This file was then analysed in Fstat 2.9.2.3 (Goudet, 1995) to determine the degree to which such an adjustment affected the results. BOTTLENECK (version 1.2.02;

Cornuet & Luikart, 1996) was used to estimate the degree of departure from mutation-drift equilibrium from the difference in expected and observed heterozygosity under the infinite allele, stepwise mutation, and two phase mutation models.

To estimate the degree of genetic structure among the sampled populations Wrights F-statistics, including F_{is} , F_{it} , F_{st} , and pairwise F_{st} (according to Weir & Cockerham, 1984) were estimated with MSA (Dieringer & Schlotterer, 2003). MSA (Dieringer & Schlotterer 2003) was also used to test the significance of the global estimate of F_{st} through 1000 permutations relaxing the assumption of Hardy-Weinberg equilibrium. Nei's unbiased genetic distance (1978) was calculated across all loci using POPDIST (Guldbrandtsen et al. 2000). From the pairwise distance matrix, MEGA version 2.1 (Kumar et al. 2001) was used to create a Neighbor-Joining Tree. Isolation by distance was estimated in GENEPOP (Raymond & Rousset, 1995) using $F_{st}/(1 - F_{st})$ and a rough pairwise matrix of the Euclidean distances between populations.

In order to estimate gene flow between the sampled populations, the coalescent-based program MIGRATE was used (Beerli & Felsenstein, 1999; 2001). N_m (the number of migrants per generation) was also estimated from pairwise F_{st} values using the method of Slatkin (1985) in GENEPOP (Raymond & Rousset, 1995). It has been shown that conclusions from estimates of migration based on F_{st} can be problematic and unreliable (e.g. Waples, 1998; Whitlock & McCauley, 1999), and will therefore only be used for comparative purposes between the two methods. However, statistics based on coalescent theory and gene genealogies are not without caveats. An appreciable number of loci is

needed in order to decrease the noise inherent in the coalescent process. MIGRATE (Beerli & Felsenstein, 1999; 2001) uses an island model but allows for asymmetric gene flow and unequal effective population sizes. In the analyses it was assumed that equal mutation rates among the loci and mutations accumulate according to the Brownian motion model. The assumption of equal mutation rates may be an unrealistic assumption (Chakraborty et al. 1997), but avoids over-parameterisation of the model and reduces the computational run time. The analysis strategy was set to maximum likelihood and the settings for the Markov Chain Monte Carlo (MCMC) sampler included recording 500 of 10,000 sampled genealogies from 10 short chains followed by three long chains sampling 1,000,000 genealogies while recording 5000. The first 10,000 trees were discarded as “burn-in” in each long chain. A calculation of F_{st} was used as the initial value of Θ ($4Ne\mu$) and a random tree was used as the starting genealogy. To ensure that parameter space had been adequately explored multiple runs were done as described above. The parameter estimates associated with the run that had the best maximum likelihood estimate among the runs are presented in the results.

Results

The total number of individuals analysed for each locus ranged from 124–137, and the number of alleles per locus among the four microsatellite loci ranged from 10–25. The minimum number of sampled individuals from a population was 9 and the maximum was 47 (Table II). The low sample size of a few populations may increase the variance associated with the frequency based statistics. Repeat unit length among loci varied from 8–30 units. The observed and expected heterozygosity ranged from 0.111–0.988 and 0.413–0.916, respectively. The observed heterozygosity at loci *Hel-10* and *Hel-8* in the Northern Taiwan population and at locus *Hel-8* in the Southern Taiwan populations was the lowest and showed a significant level of heterozygote deficiency (Table II). The highest level of heterozygosity was found at locus *Hel-8*, which had 25 alleles and showed a significant level of heterozygote deficiency in four of the seven populations.

Comparisons of observed and expected heterozygosity revealed a total of ten cases where a locus deviated significantly from Hardy-Weinberg expectations (Table II), all of which were in the direction of heterozygote deficiency. Deviations from Hardy-Weinberg equilibrium may represent the presence of null alleles or violation of one or more of the Hardy-Weinberg assumptions. Because null alleles have been reported in the literature for microsatellites (e.g. Ball & Chapman, 2003), Brookfield’s

method (1996) was used to estimate the frequency of null alleles assuming that all of the deficiencies observed were due solely to null alleles. The estimates of null alleles at loci in populations with significant deviations from Hardy-Weinberg expectations range from 7.6% for locus *Hel-16* in Hawaii to 28.9% for locus *Hel-16* in Northern Taiwan. A test for strong effects of null alleles using Micro-Checker (van Oosterhout et al. 2004) produced 10 locus-by-population instances where alternative genotypes were produced. The subsequent analysis utilising these adjusted genotypes in Fstat version 2.9.3.2 (Goudet, 1995) showed minor differences in population estimates that did not alter the interpretation of the results.

Deviation from Hardy-Weinberg expectations may also be reflected in transient states of linkage disequilibrium if a population is not in mutation-drift or migration-drift equilibrium. A test for gametic disequilibrium for the four microsatellite loci was significant ($p < 0.05$) in five of the six pairwise comparisons. Populations from Hawaii and Australia showed the least degree of linkage disequilibrium whereas the Northern Taiwan population showed a considerable degree of non-random association of alleles. Results from the BOTTLENECK analyses suggest that some of the microsatellite loci within populations show homozygosity excess or deficiency, but neither the p -value nor the mode shift tests were significant, indicating that the distribution of alleles was consistent with a neutral equilibrium model in each population.

Microsatellite analysis of global population structure and migration

The fixation index for *Hel-3* and *Hel-10* was significant ($F_{st} = 0.083$ and $F_{st} = 0.053$, respectively; $p < 0.05$), and these two loci contributed the most to the observed genetic structure and the overall fixation index ($F_{st} = 0.09$; Table III). The estimates of pairwise F_{st} values ranged from 0.02 (Hawaii-Australia) and 0.213 (Tunisia-N. Taiwan), however, many were < 0.05 which suggests that most of the populations were quite similar genetically (Table IV). Estimates of pairwise F_{st} also illustrate strong differentiation of the N. and S. Taiwan populations, with all pairwise F_{st} values greater than 0.141, which may also be driving the significant value of the global estimate of F_{st} . Although they should be viewed with caution, the estimates of the migration rate N_m also suggest that gene flow is occurring between all populations except the two Taiwan populations, which are also not exchanging migrants with one another (Table IV). A test of genotypic differentiation, where the null hypothesis is that the distribution of genotypes between pairs of populations at a specific

Table II. Microsatellite genetic diversity of *H. elegans*.

Locus, parameters	Sampling sites							
	Hawaii	Australia	Florida	Singapore	Tunisia	N. Taiwan	S. Taiwan	All
<i>H.el 3</i>								
<i>N</i>	22	20	46	9	12	9	12	130
<i>R</i>	201–219	198–210	198–219	201–213	198–240	201–219	195–225	195–240
<i>H_{obs}</i>	0.591*	0.450	0.543	0.444	0.583**	0.667	0.416*	0.301
<i>H_{exp}</i>	0.761	0.599	0.554	0.747	0.847	0.525	0.750	0.683
<i>r</i>	0.097	0.093	0.007	0.173	0.143	0.000	0.190	0.100
No. of alleles	7	4	8	5	9	5	5	14
Unique alleles	0	0	0	0	4	0	1	–
<i>F_{is}</i>	0.246	0.272	0.030	0.453	0.350	–0.215	0.488	0.166
<i>H.el 8</i>								
<i>N</i>	23	25	47	9	12	9	12	137
<i>R</i>	120–192	120–192	119–192	120–186	150–189	129–183	126–174	120–192
<i>H_{obs}</i>	0.565	0.600	0.787	0.778	0.833	0.556	0.167*	0.588
<i>H_{exp}</i>	0.662	0.519	0.772	0.586	0.740	0.586	0.413	0.611
<i>r</i>	0.058	0.000	0.000	0.000	0.000	0.019	0.174	0.036
No. of alleles	10	9	14	6	7	6	4	23
Unique alleles	0	0	1	1	0	3	1	–
<i>F_{is}</i>	0.167	–0.134	–0.009	–0.273	–0.084	0.111	0.624	0.025
<i>H.el 10</i>								
<i>N</i>	23	17	42	9	12	9	12	124
<i>R</i>	129–144	126–147	126–150	141–147	138–144	138–162	135–144	126–162
<i>H_{obs}</i>	0.478	0.529	0.558*	0.556	0.333	0.111*	0.988	0.412
<i>H_{exp}</i>	0.617	0.613	0.650	0.611	0.486	0.438	0.734	0.593
<i>r</i>	0.086	0.052	0.056	0.034	0.103	0.227	0.000	0.080
No. of alleles	6	5	7	3	3	4	4	10
Unique alleles	2	0	1	0	0	0	1	–
<i>F_{is}</i>	0.246	0.165	0.154	0.111	0.353	0.771	–0.313	0.275
<i>H.el 16</i>								
<i>N</i>	23	20	43	9	12	9	12	128
<i>R</i>	103–151	103–148	100–136	100–145	103–148	109–121	103–133	100–151
<i>H_{obs}</i>	0.739*	0.900	0.744**	0.556**	0.917	0.111**	0.917	0.391
<i>H_{exp}</i>	0.882	0.880	0.916	0.895	0.924	0.562	0.858	0.845
<i>r</i>	0.076	0.000	0.090	0.179	0.004	0.289	0.000	0.091
No. of alleles	14	15	15	11	12	4	9	25
Unique alleles	1	1	2	1	0	0	1	–
<i>F_{is}</i>	0.183	0.006	0.199	0.429	0.051	0.822	–0.025	0.177
Average number of alleles	9.25	8.25	10.75	6.25	7.75	4.75	5.5	

N = number of individuals; *R* = range of allele sizes; *H_{obs}* = observed heterozygosity with HWE significance indicators (**p* < 0.05; ***p* = 0.01); *H_{exp}* = expected heterozygosity; *r* = frequency of null alleles; total number of alleles; number of unique alleles and fixation index (*F_{is}*) for each locus in each population (Weir & Cockerham, 1984) including significance level from 1000 permutations (**p* < 0.05; ***p* = 0.01).

Table III. Calculations of *F*-statistics according to Weir and Cockerham (1984) at four microsatellite loci and the average of all loci (**p* < 0.05) for *H. elegans*.

Locus	<i>F_{is}</i>	<i>F_{st}</i>	<i>F_{it}</i>
<i>Hel-3</i>	0.2016 ± 0.088*	0.083 ± 0.038*	0.268 ± 0.087*
<i>Hel-8</i>	0.019 ± 0.058	0.120 ± 0.095	0.140 ± 0.128
<i>Hel-10</i>	0.175 ± 0.077*	0.053 ± 0.022*	0.218 ± 0.078*
<i>Hel-16</i>	0.179 ± 0.053*	0.0150 ± 0.022	0.190 ± 0.063*
Mean	0.151 ± 0.039*	0.090 ± 0.04*	0.227 ± 0.018*

locus is identical, revealed variation among the populations. The results suggest that Hawaii, Australia, and Singapore have a similar distribution of

genotypes at all loci. In contrast, the other populations show a degree of dissimilarity in the genotypic distribution between them. A Neighbor-Joining tree using Nei's distance measure (1978) also suggests that S. Taiwan is quite isolated followed by N. Taiwan. The algorithm also clusters Singapore and Tunisia together, which had a pairwise *F_{st}* value of 0.051. A test to determine isolation by distance among the populations using a distance matrix of pairwise *F_{st}* values between populations was not significant.

The results of the MIGRATE analysis suggest an appreciable amount of gene flow between many of the populations (Table V). The high levels of immigration and emigration of the Florida populations

Table IV. Pairwise estimates of F_{st} between populations of *H. elegans* (theta of Weir & Cockerham, 1984) (above diagonal) and estimates of N_m between populations (calculated according to Slatkin, 1985) (below diagonal).

	Hawaii	Australia	Florida	Singapore	Tunisia	N. Taiwan	S. Taiwan
Hawaii	–	0.021	0.027	0.025	0.085	0.167	0.182
Australia	14.02	–	0.036	0.043	0.142	0.178	0.211
Florida	8.23	6.80	–	0.035	0.088	0.141	0.182
Singapore	23.79	8.85	10.13	–	0.051	0.189	0.195
Tunisia	2.98	1.87	3.38	7.80	–	0.213	0.163
N. Taiwan	1.33	1.23	1.55	1.19	1.01	–	0.183
S. Taiwan	1.20	1.02	1.17	1.14	1.29	1.16	–

Table V. *Hydroides elegans*. Maximum likelihood estimates of M ($4N_e m / 4N_e \mu = m/\mu$) between pairs of populations of *H. elegans*.

	Hawaii	Australia	Florida	Singapore	Tunisia	N. Taiwan	S. Taiwan
Hawaii		4.89	4.92	2.17	7.40	3.12	4.17
Australia	3.55		3.21	1.83	1.14	3.67	2.16
Florida	40.13	23.12		21.11	28.61	38.93	37.72
Singapore	9.82	12.17	8.33		0.85	12.17	4.70
Tunisia	4.25	1.63	3.80	2.37		5.82	3.89
N. Taiwan	1.70	1.62	1.33	2.18	4.15		0.33
S. Taiwan	1.86	0.53	0.88	0.55	1.02	0.49	

The populations in the rows receive immigrants from those in the columns. For example, the estimate of the number of migrants from Hawaii entering Australia is 3.55 and in the opposite direction (individuals from Australia into Hawaii) the number of migrants is 4.89.

appeared to be the result of excessively high values of parameter estimates associated with locus *Hel-10*. Deciphering whether or not these estimates are ‘real’ or the result of noise in the coalescent process is difficult. An analysis coding the genotypes for *Hel-10* of the Florida samples as missing data did not affect the overall trend of the analyses. The results of the MIGRATE analysis also confirm the interpretation from estimates of pairwise F_{st} values, i.e. there is strong differentiation of the two Taiwan populations from one another. However, the MIGRATE analysis suggests that the two Taiwan populations are not strongly isolated from the other populations (Table V). Estimates of migration among all the other populations indicated appreciable amounts of gene flow.

Discussion

In an attempt to estimate the degree of genetic differentiation and gene flow among seven populations of *H. elegans*, four highly polymorphic microsatellite loci were analysed. Multiple methods of analyses were used, and the congruence in the signal among them suggests that sampled populations of *H. elegans* are genetically similar and experiencing an appreciable amount of gene flow despite the relatively limited larval dispersal potential of *H. elegans*. A low, but significant degree of overall genetic structure was observed and such a low value of F_{st}

(0.090) suggests that there is not a strong isolation and population differentiation within this system. From estimates of pairwise F_{st} values, it is plausible that the genetic dissimilarity of the two Taiwan populations is driving this statistical significance. Levels of heterozygosity and the number of alleles were lowest in the S. Taiwan and N. Taiwan populations. These populations may thus receive fewer immigrants relative to other populations. This is supported by the MIGRATE analysis which suggests that the Taiwan samples have an asymmetric migration rate where emigration is greater than immigration in these two populations. Estimates of pairwise F_{st} and migration between Singapore, Hawaii, Australia, Tunisia, and Florida show considerably less genetic differentiation and higher levels of migration, respectively, which is also suggested by the unrooted Neighbor-Joining tree (Figure 1) based on Nei’s genetic distance (1978).

While multiple analyses of the sampled loci suggest an additional mechanism of dispersal besides the movement of larvae, there are a few caveats. First, the location of the sampled populations used in this study may capture to some degree the global distribution of *H. elegans*, but it lacks a finer scale sampling design in addition to missing populations between those sampled. Second, it is becoming apparent that large numbers of microsatellites and samples may be necessary to accurately infer genetic relatedness among populations that have diverged

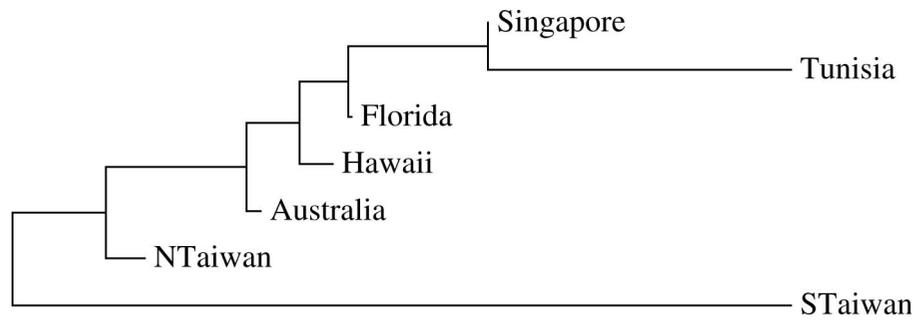


Figure 1. Neighbor Joining Tree from a distance matrix based on Nei's estimate of genetic distance (1978) for *H. elegans*.

recently (Goldstein & Pollock, 1997). For coalescent-based methods, the sampling emphasis is placed on the number of loci; as the number of loci increases the variance in estimates of migration decreases. Although in this study the number of individuals in certain populations and the number of loci may be low, this limitation has been overcome by doing multiple analyses and looking for concordance among them. Each of the analyses has demonstrated an appreciable amount of gene flow and little population structure in this system.

A relatively random pattern of deviations from Hardy-Weinberg expectations was observed. Such departures from Hardy-Weinberg equilibrium can reduce the confidence of conclusions based on F -statistics. Deviations from Hardy-Weinberg equilibrium are not uncommon when using highly polymorphic microsatellites and have been reported in previous work involving marine organisms (e.g. Roques et al. 2001; Daemen et al. 2001; Miller & Howard, 2004). Whether the departures may be explained by the presence of null alleles or small allele dominance (Wattier et al. 1998) is difficult to conclude. Null alleles occur when a primer site has a polymorphism that prevents amplification of an allele (e.g. Ball & Chapman, 2003) and small allele dominance refers to the bias in genotyping and sequencing towards the identification of smaller alleles (Wattier et al. 1998). While it may be possible to determine the frequency of null alleles by designing alternative primers, this process is difficult, time consuming, and insufficient genetic material was available to do so. The random patterns of deviations from Hardy-Weinberg equilibrium, and the random patterns of linkage disequilibrium among the microsatellite loci suggest that the populations may not be in mutation-drift or migration-drift equilibrium. Populations may not be in mutation-drift or migration-drift equilibrium if they are of recent origin, there are complicated migration patterns among populations, or fluctuations in population size have occurred. Each of these explanations is consistent with anthropogenic modes

of dispersal *via* biofouling (Lewis et al. 2006), as is the lack of isolation by distance observed.

The lifespan of *H. elegans* larvae makes natural dispersal by larvae across entire ocean basins unlikely. In addition, adult worms are restricted to coastal environments on hard substrata, which does not readily permit 'leap-frogging' across ocean basins. For marine species, the role of ocean currents may often be an influential mechanism of dispersal, but given the vast distances between our sampled populations and the relatively low dispersal potential of larvae, it does not seem plausible that ocean currents are responsible for the minimal population structure and high migration rates observed. Both the F_{st} values and the MIGRATE results allude to an additional mechanism of dispersal, namely hull fouling. Moreover, the locations of the sampled populations and current shipping routes also suggest that *H. elegans* is being dispersed *via* biofouling. Four of the sampled populations are from the top 50 busiest ports in the world (Zachail & Heideloff, 1999) measured in TEU (20-ft equivalent units; a measure of container traffic, which we assume to be correlated with shipping traffic). Singapore is the world's largest port and resides on the busiest shipping lane through the shallow Strait of Malacca (Zachail & Heideloff, 1999), which may explain its relatedness to other populations. An astounding 25% of all commercial shipping traffic passes through the Strait of Malacca annually (The Economist, 2004). The Taiwan samples are from the third busiest harbour (S. Taiwan's Kaohsiung Harbor) and the 27th busiest harbour (N. Taiwan's Keelung Harbor). This observation may explain the estimates of migration, but it also contradicts the degree of genetic differentiation inferred from pairwise F_{st} values of these two populations from the others. The degree of isolation and population differentiation between the two Taiwan samples may reflect minimal maritime traffic given that terrestrial routes are likely to be the primary avenues of distribution between the two locations. The Tunisia population is within the congested Mediterranean Sea where

H. elegans is the dominant biofouler (Koçak et al. 1999) and there is a large degree of shipping through a narrow confined waterway (Zachail & Heideloff, 1999). The Florida population is also well connected to the shipping lanes with the close proximity of Tampa Bay, which is the 44th busiest port. The sampled individuals from Australia, where *H. elegans* is a dominant biofouler (Lewis et al. 2006), and Hawaii (with Pearl Harbor) also came from busy ports, which may explain their lack of genetic differentiation from other populations despite their somewhat geographically isolated locations.

The analysis of four microsatellites suggests *H. elegans* is being transported in appreciable numbers *via* an anthropogenic dispersal mechanism, i.e. through hull fouling and likely to a lesser degree, ballast water. Although the number of loci and individuals from certain populations was somewhat low, the consistency between separate analytical methods used to estimate population structure suggests a true pattern of high migration and gene flow among populations separated by vast distances. The study illustrates the utility of molecular markers for estimating gene flow by human-mediated dispersal mechanisms, which can have important ecological and evolutionary consequences for community structure.

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