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Genetic differentiation of populations of the common intertidal nemerteans *Lineus ruber* and *Lineus viridis* (Nemertea, Anopla)

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Abstract

Specimens of the common intertidal nemerteans *Lineus ruber* and *L. viridis* were collected from sites along the west and Southwest coasts of Britain, northern France and North America. Allele frequencies of up to 13 putative enzyme loci were estimated for all populations of *L. ruber* and *L. viridis*. Estimates of genetic variation were low for populations of *L. ruber* (H_{obs} 0.008–0.052) but were higher for populations of *L. viridis* (H_{obs} 0.068–0.153). Exact tests for conformity of observed genotype frequencies to those expected under Hardy-Weinberg equilibrium failed to detect significant deviations for *L. ruber* or *L. viridis*. *F*-statistics were affected by small sample size and low expected values in some populations, but, F_{ST} was significantly different from zero for most loci examined for both *Lineus ruber* and *L. viridis*. This indicated a significant degree of population structuring for both species (only a moderate level of gene-flow). Intraspecific comparisons of genetic distance and genetic identity showed little evidence of genetic differentiation between populations separated by large geographic distances (1000s of km). There was no apparent relationship between genetic distance between populations and the geographic distance separating them. Possible explanations for this lack of genetic differentiation between populations of *L. ruber* and *L. viridis* are discussed. These include a lack of variation in the enzyme loci sampled caused by population dynamics, balancing selection in the enzyme loci sampled, large introductions between populations and passive dispersal.

Introduction

The anoplan nemerteans *Lineus ruber* (Müller, 1774) and *L. viridis* (Müller, 1774) are commonly found in a variety of intertidal habitats on the coasts of northern Europe and North America (Gibson, 1982; Nordhausen, 1988; Thiel & Reise, 1993). *L. ruber* has also been reported from the Mediterranean, the North Sea, the Pacific coast of North America, Madeira, Greenland, Iceland, the Faroes, Siberia and South Africa (Gibson, 1982). The vertical distribution of these two species appears to vary according to habitat availability and season (Rogers et al., 1995). Both species are predators and feed upon a variety of polychaetes, crustaceans and molluscs (McDermott & Roe, 1985; Nordhausen, 1988; Thiel & Reise, 1993).

Lineus ruber generally reproduces sexually in late winter to late spring. The exact time varies according to geographic locality. In the Gulf of Maine it is reported to reproduce in March/April (Coe, 1899; Riser, 1974), in the Barents sea July–August (Schmidt & Jankovskaia, 1938) and in the English Channel, December–January (Oxner, 1911; Gontcharoff, 1951; Bierne, 1970). Under laboratory conditions the sea-

sonality of reproduction in *L. ruber* disappears rapidly (Bierne, 1970; Vernet & Bierne, 1993). It is therefore likely that environmental factors influence the timing and length of the reproductive cycle in this species (Vernet & Bierne, 1993).

During reproduction in *Lineus ruber*, a male and female enclose themselves in a gelatinous mucous cocoon, secreted by the female and firmly attached to a rock (Gontcharoff, 1951). The male releases sperm of a modified type (Franzén, 1983) and these fertilise the eggs that are released into the mucus cocoon. Gontcharoff (1951) observed sperm in the gonoducts and ovaries of *L. ruber* which probably act as receptacula seminalis (Cantell, 1975). Reproduction in *L. ruber* may be regarded as a form of pseudocopulation (Riser, 1974; Franzén, 1983). The adults leave the cocoon after reproduction and die 2–3 weeks later (Riser, 1974).

Lineus ruber has encapsulated larvae (Desor larva) of which only 12–13% hatch within the mucus cocoon (Schmidt, 1946) (approximately 10–15 larvae according to Gibson [1972]). The remaining larvae/eggs are eaten by the hatchlings which then leave the cocoon as miniature worms (Schmidt, 1946; Gontcharoff, 1951; Gibson, 1972; Bierne, 1983). The juvenile worms feed immediately on leaving the cocoon and initially show a weak positive or more usually indifferent response to light that rapidly develops into a strong negative phototaxis (Gontcharoff, 1951).

Lineus viridis shows a similar type of reproduction to *L. ruber* but larval development in the two species is different. In *L. viridis* the first larvae to hatch do not devour the rest. As a result 400–500 juvenile worms hatch from each mucus egg string. Also in contrast to *L. ruber*, juvenile *L. viridis* are strongly phototactic and do not feed for 2–3 weeks after hatching (Gontcharoff, 1951; Gibson, 1982).

Both *Lineus ruber* and *L. viridis* are small invertebrates with limited powers of locomotory dispersal as adults. Both exhibit a form of pseudocopulation and direct development with a very limited capacity for larval dispersal, though *L. viridis* is more fecund than *L. ruber*. It is conceivable that the behaviour of juvenile *L. viridis* after hatching may make it more likely that they will be carried up in to the water column than those of *L. ruber*. It would be expected that with such a life history geographically separated populations of both species would show marked genetic differentiation due to poor gene flow (see Burton, 1983; Waples, 1987; Ward, 1989; Piertney & Carvalho, 1994). Gene flow is defined as genetically effective migration, i.e. an exchange between conspecific populations of successfully fertilising gametes or individuals that survive to reproduce in the population into which they have migrated (Hedgecock, 1986).

In this study levels of genetic differentiation of geographically separated populations of *Lineus ruber* and *L. viridis* are estimated using starch gel electrophoresis of allozymes. The populations for which differentiation is estimated are located on the west coast of the Britain, northern France and the east coast of North America and are separated at spatial scales of tens to thousands of kilometres. Allozyme electrophoresis has been used previously to investigate the systematics of nemerteans (Cantell & Gidholm, 1977; Williams et al., 1983; Sundberg & Janson, 1988; Rogers, 1993; Rogers et al., 1993, 1995) but this study is the first to consider intraspecific population differentiation within the phylum.

Materials and methods

Samples

Various nemerteans were collected between 1989 and 1992 from around the west and Southwest coasts of Britain, northern France and the east coast of North America. The sites are listed in Table 1 along with the numbers of *Lineus ruber* and *L. viridis* collected from each site (see also Rogers et al., 1995 for map).

All specimens were collected intertidally beneath stones and rocks lying in silt, muddy sand, sand or fine shelly gravel. Specimens were carefully removed from the substratum and transported back to Port Erin Marine Laboratory, Isle of Man. Specimens were subsequently kept in aerated plastic tanks, in which they were starved prior to electrophoresis.

Electrophoresis

For electrophoresis 5–15 mm of tissue was removed from the posterior end of each specimen. Specimens were alive to prevent possible reduction of enzyme activity that may have resulted from using frozen tissue (Scozzani et al., 1980). The tissue was homogenised, over ice, in 100 μl of 0.06M Tris-HCl pH 8.0 using the tip of a glass rod. The homogenate was absorbed on to filter paper wicks (Whatman No. 3) and applied to a starch gel.

Horizontal starch gel electrophoresis was performed by standard methods (see Harris & Hopkinson, 1978; Murphy et al., 1990) using 12.5% starch gels (Sigma Chemical Co. Ltd, Poole, Dorset). Two buffer

Table 1. Numbers of *Lineus ruber* and *L. viridis* sampled at sites in Britain, France and North America

Site	Lat/Long	Site code	*Lineus ruber*	*Lineus viridis*
United States of America				
Mount Desert Island	44°22′N, 68°19′W	US	68	25
Canada				
Brandy Cove	45°05′N, 67°04′W	CAN	08	10
United Kingdom				
Oban	56°24′N, 05°32′W	OBN	90	48
Whitehaven	54°34′N, 03°35′W	WTN	–	34
Barrow	54°06′N, 03°16′W	BRW	–	13
Port Erin	54°05′N, 04°46′W	PTE	13	–
Bay Ny Carrickey	54°05′N, 04°41′W	BNC	–	29
Castletown	54°04′N, 04°39′W	CST	38	–
Llandudno	53°19′N, 03°49′W	LDN	55	59
Anglesey	53°18′N, 04°02′W	ANG	88	30
St. Agnes	50°20′N, 04°11′W	STA	01	118
Plymouth Sound	50°20′N, 04°09′W	PLY	–	20
Wembury	50°19′N, 04°05′W	WMY	–	27
France				
Pointe de Barfleur	49°18′N, 00°25′W	FRA	07	07

systems were used: Buffer system I, Tris-citrate, continuous, pH 8.0 (Ward & Beardmore, 1977); Buffer system II, Tris-citrate, continuous, pH 7.1 (Williams et al., 1983). Buffer system I was run at 140V, 50 mA for 7 h, buffer system II was run at 100V, 50 mA for 7 h.

Nine enzymes coding for a total of 13 loci were visualised using enzyme specific stains given in Harris & Hopkinson (1978) and Rogers et al. (1993): Aminopeptidase (AP – substrate, GLY-LEU, Sigma Chemical Co. Ltd., Poole, U. K.) E. C. 3.4.11.1; Fumarate hydratase (FH) E.C. 4.2.1.2; Glutamate-oxaloacetate transaminase (GOT) E.C. 2.6.1.1; Isocitrate dehydrogenase (ICD) E. C. 1.1.1.42; Malate dehydrogenase (MDH) E.C. 1.1.1.37; Octopine dehydrogenase (ODH) E.C. 1.5.1.11; 6-phosphogluconate dehydrogenase (PGD) E.C. 1.1.4.4; Phosphoglucose isomerase (PGI) E.C. 5.3.1.9; Phosphoglucomutase (PGM) E.C. 5.4.2.2. ICD did not stain consistently for all sites for *Lineus viridis* and so was not included in data analysis for this species.

Specimens from different populations were run simultaneously on starch gels to directly compare allele mobilities for the loci scored.

Data analysis

Prior to data analysis genetically divergent individuals representing a cryptic species were removed from the data (see Rogers et al., 1995). Mean observed and expected heterozygosities were calculated for 13 loci for *Lineus ruber* and 11 loci for *L. viridis*. Fisher's (1935) Exact Test was performed to examine the conformity of observed genotypes to those expected under Hardy-Weinberg equilibrium. Significance levels were adjusted for multiple tests using the sequential Bonferroni technique (see Rice, 1989).

F_{IS}, F_{IT} and F_{ST} (Wright, 1978) were calculated for all loci for populations of *Lineus ruber* and *L. viridis* in which the number of individuals sampled (N) equalled or exceeded 25. These were used to estimate the total amount of deviation from expected genotype frequencies in the populations investigated (F_{IT}) and the portion of this deviation due to non-random breeding (F_{IS}) and population subdivision (F_{ST}). The significance of F_{IS} and F_{ST} was calculated using the formulae given in Waples (1987). The number of migrants per generation between populations (N_em) was calculated for all populations of *L. ruber* and *L. viridis*, again according to Waples (1987). N_em indicates the average relative gene flow between all populations of each species.

Overall genetic differentiation between intraspecific populations of *Lineus ruber* and *L. viridis* was estimated using Nei's (1972) genetic identity (I) and distance (D). These calculations were performed using BIOSYS-1 version 1.7 (Swofford & Selander, 1989). A Pearson's correlation coefficient was calculated

Table 2. Allele frequencies for *Lineus ruber, Lineus viridis* for all sites (alleles are numbered in order of decreasing mobility)

Locus	Allele	*Lineus ruber*								*Lineus viridis*											
		US	CAN	OBN	PTE	CST	LDN	ANG	FRA	US	CAN	OBN	WTN	BRW	BNC	LDN	ANG	STA	PLY	WMY	FRA
Ap	1	–	–	–	–	–	–	–	–	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.071
	3	–	–	–	–	–	0.018	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	1.000	1.000	1.000	1.000	1.000	0.982	0.959	1.000	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	0.027	–	–	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	0.014	–	–	–	–	–	–	–	–	–	–	–	–	–
Fh-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Fh-2	1	–	–	–	–	–	–	–	–	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	–	–	–	–	–	–	–	–	–	–	–	–
Got-1	1	–	–	–	–	–	–	–	–	–	–	–	0.015	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	0.800	0.950	0.979	0.956	1.000	0.983	1.000	0.983	1.000	0.950	0.905	1.000
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–	–	–
	4	0.022	–	–	–	–	0.014	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.025	0.095	–
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–	–	–	–	–
	7	–	–	–	–	–	–	–	–	0.200	0.050	0.021	–	–	–	–	–	–	0.025	–	–
	8	0.978	1.000	1.000	1.000	1.000	0.986	1.000	1.000	–	–	–	0.029	–	–	–	–	–	–	–	–
Got-2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	–
Icd-1	1	–	–	–	–	0.013	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	1.000	1.000	0.986	1.000	0.961	1.000	0.994	1.000	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	0.014	–	0.026	–	0.006	–	–	–	–	–	–	–	–	–	–	–	–	–
Icd-2	1	1.000	1.000	1.000	1.000	1.000	1.000	0.989	1.000	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	0.011	–	–	–	–	–	–	–	–	–	–	–	–	–
Mdh-1	1	–	–	–	–	–	–	–	–	–	–	0.073	–	–	0.052	–	–	0.008	0.050	0.024	–
	2	–	–	–	–	–	–	–	–	0.938	1.000	0.917	1.000	0.962	0.621	0.754	0.983	0.983	0.925	0.952	1.000
	3		–	–	0.077	–	–	–	0.071	0.063	–	0.010	–	0.038	0.328	0.246	0.017	0.008	0.025	0.024	–
	4	1.000	1.000	0.989	0.846	1.000	1.000	1.000	0.929	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	0.011	0.038	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	0.038	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mdh-2	1	–	–	–	–	–	–	–	–	0.938	1.000	1.000	1.000	1.000	1.000	0.992	0.983	1.000	1.000	1.000	1.000
	2	–	–	–	–	–	–	–	–	0.063	–	–	–	–	–	0.008	0.017	–	–	–	–
	3	–	–	–	–	–	–	–	0.143	–	–	–	–	–	–	–	–	–	–	–	–
	4	1.000	1.000	0.989	1.000	1.000	0.991	1.000	0.857	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	0.011	–	–	0.009	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 2. (continued)

Locus	Allele	*Lineus ruber*								*Lineus viridis*											
		US	CAN	OBN	PTE	CST	LDN	ANG	FRA	US	CAN	OBN	WTN	BRW	BNC	LDN	ANG	STA	PLY	WMY	FRA
Odh	1	–	–	–	–	–	–	–	–	0.053	–	–	–	–	–	–	0.233	–	–	–	–
	2	–	–	–	–	–	–	–	–	0.079	0.063	0.346	0.146	0.115	–	0.529	0.033	0.112	–	0.029	0.143
	3	–	–	0.067	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	0.125	0.135	–	–	–	0.114	0.200	0.009	–	0.029	–
	5	–	0.063	0.094	–	0.132	0.182	–	0.071	0.316	0.188	0.500	0.292	0.154	0.138	0.171	0.200	0.543	0.150	0.353	0.286
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	0.017	–	–	–	–	–	0.143
	7	0.993	0.875	0.839	1.000	0.868	0.818	0.994	0.929	–	–	–	–	–	–	–	–	–	–	–	–
	8	–	–	–	–	–	–	–	–	0.474	0.188	0.019	0.417	0.423	0.517	0.171	0.267	0.310	0.275	0.500	0.071
	9	–	–	–	–	–	–	–	–	–	0.125	–	–	–	–	–	–	–	–	–	0.286
	10	–	–	–	–	–	–	–	–	0.079	0.125	–	0.146	0.308	0.328	0.014	–	0.017	0.550	0.059	0.071
	11	0.007	0.063	–	–	–	–	0.006	–	–	–	–	–	–	–	–	0.067	–	–	–	–
	12	–	–	–	–	–	–	–	–	–	0.188	–	–	–	–	–	–	0.009	0.025	0.029	–
Pgd	1	–	–	–	–	–	–	–	–	–	0.050	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	0.958	0.900	1.000	1.000	1.000	1.000	1.000	1.000	0.987	1.000	1.000	1.000
	3	–	–	–	–	–	–	–	–	0.042	–	–	–	–	–	–	–	0.008	–	–	–
	4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	–	0.050	–	–	–	–	–	–	0.004	–	–	–
Pgi	1	–	–	–	–	0.013	–	–	–	–	–	–	–	–	–	0.025	–	0.008	–	0.048	–
	2	0.007	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.004	–	–	–
	4	–	–	–	–	0.039	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	0.983	1.000	0.952	1.000
	6	0.993	1.000	1.000	1.000	0.947	1.000	1.000	1.000	–	–	–	–	–	–	–	–	–	–	–	–
	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.004	–	–	–
Pgm	1	–	–	–	–	–	–	–	0.071	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.016	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	0.063	–	–	–	–	–	0.026	–	0.043	–
	4	0.007	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	0.007	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	0.596	1.000	0.927	0.912	1.000	0.845	0.958	0.800	0.719	0.900	0.717	0.857
	7	0.007	–	–	–	–	0.027	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	–	–	–	–	–	–	–	–	0.404	–	0.010	0.088	–	0.155	0.042	0.200	0.240	0.050	0.196	0.143
	9	0.985	1.000	0.986	0.962	0.987	0.973	0.994	0.857	–	–	–	–	–	–	–	–	–	–	–	–
	10	–	–	–	–	0.013	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	11	–	–	–	–	–	–	–	0.071	–	–	–	–	–	–	–	–	–	–	–	–
	12	–	–	–	0.038	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.043	–
	14	–	–	–	–	–	–	0.006	–	–	–	–	–	–	–	–	–	–	–	–	–
	15	–	–	0.007	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.050	–	–

between Nei's (1972) D between populations and geographic distance separating populations for *L. viridis* and *L. ruber* using the programme Minitab (Minitab, 1989).

Results

Allele frequencies for all enzyme loci that produced well-resolved staining patterns consistent with known subunit structures for all populations of *Lineus ruber* and *L. viridis* are given in Table 2. Allele frequencies are very similar for intraspecific comparisons but are significantly different between the two species (see Rogers et al., 1993, 1995). Observed heterozygosities, which ranged from 0.008–0.052 (Table 3) were generally low for *L. ruber* compared with those recorded for other eukaryotes (see Nevo, 1978; Nevo et al., 1984). Note that the highest of these values came from the French site that had a very low sample size (7 individuals). Higher observed heterozygosities were recorded for populations of *L. viridis* with a range of 0.068–0.153. This range is comparable to that found in other eukaryotes (Nevo, 1978; Nevo et al., 1984).

Fisher's (1935) Exact Test for deviation from Hardy-Weinberg expectations, with significance levels adjusted for multiple tests, showed no significant deviations from expected genotype frequencies for populations of *Lineus ruber* or *L. viridis*.

F-statistics for loci variable across all populations for *Lineus ruber* and *L. viridis* in which $N \geq 25$, are given in Table 4. For *L. ruber* two out of nine loci showed highly significant F_{IS} values. In both of these loci there was one common allele and 1–3 rare alleles. This may have led to small expected values in tests of significance for F_{IS}, especially for *Pgi* but may also indicate a degree of inbreeding within populations. Mean F_{IS} for *L. ruber* was not significant.

F_{ST} values for populations of *L. ruber* were highly significant for five out of nine variable loci and significant overall. The number of migrants per generation between populations (N_em) indicated a moderate level of gene flow between populations. However, as with F_{IS}, significance of F_{ST} was likely to have been influenced by low expected frequencies due to low genetic variation and low sample sizes in some populations. The only locus that showed substantial variation in *L. ruber* was *Odh* and it is notable that F_{ST} was highest for this enzyme locus. It is therefore likely that the degree of population structuring indicated by F_{ST} is underestimated.

Although the genetic variability (mean heterozygosity) of the enzyme loci scored for *L. viridis* was higher than in *L. ruber* (especially for *Odh*), most enzyme loci still consisted of a single common allele with several rare alleles. For populations of *L. viridis* F_{IS} values were significant for *Got-1*, *Mdh-1* and *Pgm* but the overall F_{IS} value was not significant. Most F_{ST} values for *L. viridis* were highly significant, as was the overall F_{ST} value. Though the significance of F_{ST} values was probably influenced by low expected frequencies for rare alleles and low sample sizes at some sites, this will have been offset to some degree by the low variability of the loci studied (leading to an overestimate of gene flow). These results therefore do indicate a degree of population structuring in *L. viridis*. The number of migrants per generation between populations was lower in *L. viridis* than in *L. ruber*; this is probably the result of the difference in variability of the loci studied between the two species.

Nei's (1972) genetic identity between populations of *Lineus ruber* is very high with a range of 1.000 to 0.996 (see Table 5). This lack of genetic differentiation is at least partially due to the low variability of the loci studied. Genetic identity and distance estimates for *L. viridis* suggest more structured populations for this species, but there is still very little genetic divergence across the geographic range sampled ($I = 0.957$–0.995, $D = 0.003$–0.044; see Table 6). The correlation coefficient of genetic distance between populations and geographic distance separating populations indicated little relationship between these parameters (*L. ruber* $r = -0.366$; for *L. viridis* $r = 0.009$).

Discussion

Fisher's (1935) Exact Test on populations of *L. ruber* and *L. viridis* failed to detect any significant deviations from genotype frequencies expected under Hardy-Weinberg equilibrium. Although this test takes into account small sample size, it does so by pooling genotype frequencies and may therefore underestimate the significance of deviation from Hardy-Weinberg expectations (Lessios, 1992).

F_{IS} values for *Lineus ruber* were affected by small sample size and low variability in the enzyme loci studied. Overall F_{IS} values for *L. viridis* were not significant.

F_{ST} values for *Lineus ruber* gave highly significant results for over half the loci examined. The mean F_{ST} value was higher than that found over similar geographic distances in several molluscan species with

Table 3. Mean observed and expected heterozygosity (under Hardy-Weinberg expectations) for populations of *Lineus ruber* and *L. viridis*. Mean sample size per locus and percentage of polymorphic loci (at 0.95 and 0.99 levels) are also given

Population	Mean sample size per locus	Percentage polymorphic loci (95%)	Percentage polymorphic loci (99%)	Mean observed heterozygosity	Mean expected heterozygosity
L. ruber					
US	65.1	0.0	23.1	0.008	0.008
CAN	8.0	7.7	7.7	0.019	0.019
OBN	80.0	7.7	38.5	0.029	0.032
PTE	13.0	7.7	15.4	0.018	0.028
CST	38.0	15.4	30.8	0.031	0.038
LDN	45.2	9.1	45.5	0.036	0.040
ANG	70.7	0.0	15.4	0.009	0.009
FRA	7.0	30.7	30.7	0.052	0.075
L. viridis					
US	22.4	45.5	54.5	0.153	0.165
CAN	9.8	27.3	27.3	0.118	0.109
OBN	46.0	30.8	38.5	0.074	0.087
WTN	33.1	18.2	27.3	0.068	0.088
BRW	13.0	9.1	18.2	0.084	0.072
BNC	28.8	27.3	36.4	0.119	0.130
LDN	53.2	23.1	38.5	0.105	0.107
ANG	25.2	23.1	46.1	0.100	0.113
STA	107.1	18.2	45.5	0.123	0.102
PLY	18.5	36.4	36.4	0.086	0.095
WMY	18.3	27.3	45.5	0.118	0.133
FRA	7.0	27.3	27.3	0.091	0.103

long-lived planktonic larvae (Levington & Suchanek, 1978; Johnson & Black, 1984; Benzie & Williams, 1992). This suggests that, as expected, *L. ruber*, a species with direct development, shows a lower gene flow between populations than in some species with planktonic larval phases (e.g., Burton, 1983; Hedgecock, 1986; Waples, 1987; Hunt & Ayre, 1989; Ward, 1989). F_{ST} values obtained for *L. ruber* though, were not as high as those obtained for species with planktonic larvae that do show a degree of population differentiation (Smith & Potts, 1987; Macaranas et al., 1992).

F_{ST} values for *Lineus viridis* were significant at almost all loci and were higher than those found in *L. ruber*. It is initially surprising that gene flow is lower than that observed in *L. ruber*. *L. viridis* is expected to have a greater potential for dispersal than *L. ruber*; it is more fecund and its larvae are positively phototactic and therefore more likely to become suspended in the water column (see Hagerman & Rieger, 1981; Dobbs & Vozarik, 1983) . However the differences in F_{ST} between *L. ruber* and *L. viridis* are not large and are probably confounded by the low variation of allozyme loci sampled for *L. ruber* (see below).

While populations of *Lineus viridis* and *L. ruber* show some degree of differentiation, estimates of Nei's (1972) genetic distance and identity between populations show genetic cohesiveness over very large (trans-Atlantic) geographic distances. Other groups of intertidal marine invertebrates that have pelagic larvae have shown marked genetic differentiation between populations located on the east and west sides of the North Atlantic ocean. In the cirripede, *Semibalanus balanoides*, allele frequency differences at the *Pgi* and *Mpi* loci indicated the presence of two populations in the North Atlantic (Flowerdew, 1983), one extending along the coasts of Europe from Spain in the south to Spitzbergen north of Norway, the other along the east coast of the USA through to Newfoundland and Iceland. Studies on *Mytilus edulis* have shown differences

Table 4. Summary of F-statistics at all loci for populations of *Lineus ruber* and *L. viridis* in which $N \geq 25$. ** $P < 0.01$

Locus	F_{IS}	F_{IT}	F_{ST}	N_ee
Lineus ruber				
Ap	–0.028	–0.008	0.020**	
Got-1	–0.019	–0.007	0.012	
Icd-1	–0.025	–0.010	0.015**	
Icd-2	–0.011	–0.002	0.009	
Mdh-1	–0.048	–0.013	0.033**	
Mdh-2	–0.010	–0.004	0.006	
Odh	0.182**	0.236	0.065**	
Pgi	0.416**	0.434	0.030**	
Pgm	–0.017	–0.010	0.007	
Mean	0.116	0.155	0.044**	5.43
Lineus viridis				
Got-1	–0.151**	–0.035	0.100**	
Mdh-1	0.170**	0.301	0.158**	
Mdh-2	–0.051	–0.011	0.038	
Odh	0.012	0.142	0.131**	
Pgd	–0.035	–0.006	0.028**	
Pgi	–0.036	–0.010	0.025**	
Pgm	0.166**	0.240	0.089**	
Mean	0.055	0.168	0.119**	1.85

in allele frequencies for leucine aminopeptidase (*Lap*), *Ap*, *Pgi* and *Pgm* between populations located on the east and west sides of the North Atlantic (Gosling, 1992).

The population dynamics of *Lineus ruber* and *L. viridis* may explain the low genetic distances between geographically distant populations. *L. ruber* and *L. viridis* are short lived species with populations that are often small and numerically variable, over a period of years. Both these species are found generally under stones and other epibenthic materials (Gibson, 1982; Thiel & Reise, 1993) that may be disturbed frequently, especially during winter storms, possibly causing mortality of individual nemerteans and, from time to time, entire local populations (see Osman, 1977; Sousa, 1979, 1980). Other factors such as environmental temperature, predation and parasitisation may contribute to mortality in nemertean populations (Roe, 1976).

While both species investigated apparently have a large geographic range, possibly consisting of a very large number of individuals, they are divided into many smaller populations over this range; as indicated by estimates of F_{ST} in this study. These smaller populations may be affected by many extinction and recolonization events due to the nature of preferred habitat type of these species and by other factors. In such a case, extinction and recolonization will reduce the effective size and variation of the total species population and reduce the divergence of subpopulations (Wright, 1940; Maruyama & Kimura, 1980; Slatkin, 1985, 1987). This would explain the genetic cohesion of populations of these two species. What is usually interpreted as a moderate level of gene flow may in this case be a suppression of genetic divergence by extinction of local populations.

The only apparent difference in the biology of *Lineus ruber* and *L. viridis* is that the former is less fecund than the latter. It would be expected that with all other things being equal this alone would increase the extinction rate and increase the time for population recovery in *L. ruber* compared with *L. viridis*. *L. ruber* would have a smaller effective (and probably actual) population size than *L. viridis* and would therefore be expected to have a lower heterozygosity (see Kimura, 1983; Nei, 1987; Solé-Cava & Thorpe, 1991) which fits observations made in this investigation. It is interesting that the cryptic species found in very low numbers with *L. ruber* and *L. viridis* (Rogers et al., 1995) also has a very low heterozygosity.

There are several other possible factors that may contribute to genetic homogeneity between populations of *Lineus ruber* and *Lineus viridis*. There is evidence that the inference of genetic structure of natural populations from the geographic distribution of alleles derived from allozyme data is not as simple as has previously been suggested by the neutral gene theory. Studies of several species of marine animals have shown that population structure can be resolved at different levels by allozyme electrophoresis and by molecular techniques (see González-Villaseñor & Powers 1990; Burton & Lee, 1994). Furthermore, in at least one study, apparent geographic unity in allele frequencies has been contrasted with pronounced population subdivision indicated by restriction analysis of mitochondrial DNA sequences (Karl & Avise, 1992). The most likely explanation for the lack of geographic variation in allozyme frequencies in this case was thought to be balancing selection on protein coding loci counteracting genetic drift in a species with a subdivided population structure.

The apparent lack of genetic differentiation in *Lineus* populations located on either side of the Atlantic could also be explained by the introduction of large numbers of individuals of these species from one side of the ocean to the other. The lack of genetic differen-

Table 5. Matrix of Nei's (1972) genetic identity (*I*) (above diagonal) and genetic distance (*D*) (below diagonal) for all populations of *Lineus ruber*

Population	US	CAN	OBN	PTE	CST	LDN	ANG	FRA
US	–	0.999	0.999	0.999	0.998	0.998	1.000	0.997
CAN	0.001	–	1.000	0.998	0.999	0.999	0.999	0.997
OBN	0.001	0.000	–	0.997	0.999	0.999	0.998	0.997
PTE	0.001	0.002	0.003	–	0.998	0.996	0.999	0.997
CST	0.002	0.001	0.001	0.002	–	0.999	0.998	0.996
LDN	0.002	0.001	0.001	0.004	0.001	–	0.997	0.996
ANG	0.000	0.001	0.002	0.001	0.002	0.003	–	0.997
FRA	0.003	0.003	0.003	0.003	0.004	0.003	0.004	–

Table 6. Matrix of Nei's (1972) genetic identity (*I*) (above diagonal) and genetic distance (*D*) (below diagonal) for all populations of *Lineus viridis*

Population	US	CAN	OBN	WTN	BRW	BNC	LDN	ANG	STA	PLY	WMY	FRA
US	–	0.971	0.965	0.985	0.975	0.974	0.961	0.984	0.989	0.970	0.992	0.973
CAN	0.030	–	0.984	0.991	0.991	0.972	0.978	0.988	0.981	0.985	0.983	0.990
OBN	0.036	0.016	–	0.985	0.977	0.957	0.987	0.981	0.987	0.968	0.976	0.987
WTN	0.015	0.009	0.015	–	0.997	0.982	0.981	0.990	0.992	0.988	0.995	0.988
BRW	0.025	0.009	0.023	0.003	–	0.987	0.979	0.985	0.981	0.994	0.987	0.983
BNC	0.026	0.028	0.044	0.018	0.013	–	0.971	0.974	0.971	0.984	0.981	0.966
LDN	0.040	0.022	0.013	0.019	0.021	0.030	–	0.976	0.972	0.966	0.970	0.977
ANG	0.016	0.012	0.019	0.010	0.015	0.027	0.025	–	0.988	0.977	0.990	0.986
STA	0.011	0.019	0.013	0.008	0.019	0.030	0.028	0.012	–	0.973	0.995	0.986
PLY	0.030	0.015	0.032	0.012	0.006	0.016	0.035	0.023	0.027	–	0.979	0.977
WMY	0.008	0.017	0.024	0.005	0.013	0.019	0.031	0.010	0.005	0.021	–	0.981
FRA	0.027	0.010	0.013	0.012	0.018	0.035	0.023	0.014	0.014	0.023	0.019	–

tiation between populations of the cirripede *Elminius modestus* in Australia, New Zealand and Europe is thought to be due to such introductions (Dando, 1987). Large introductions of *L. ruber* and *L. viridis* might have taken place by transport with other organisms, such as cultivated bivalves (see Christiansen & Thomsen, 1981), or as a component of fouling communities of ships (see Bertelsen & Ussing, 1936; Carlton, 1985). Passive dispersal by rafting may also maintain a moderate level of gene flow between geographically distant populations (see Highsmith, 1985; Edgar, 1987; Jokiel 1989; DeVantier, 1992). The low level of gene flow maintained by such means may be all that is required to prevent genetic divergence between populations of *L. ruber* and *L. viridis* (see Wright, 1940; Kimura, 1955; Slatkin, 1985; Maynard Smith, 1989).

To summarise, the analysis of population structure in *Lineus ruber* and *L. viridis* has detected a moderate level of genetic differentiation between populations at different spatial scales. This genetic differentiation appears to be unrelated to geographic separation of the populations studied. Many studies of genetic differentiation, in populations of marine invertebrates, have shown that the dispersal potential of larvae is often not realised (e.g., Todd et al., 1988). Few studies have shown genetic homogeneity over large distances where little dispersal is expected from examination of the life history of the species under investigation (e.g., Solé-Cava et al., 1985). The latter seems to be the case in the present study but genetic differentiation has probably been underestimated due to the low variation of the loci detected or due to an inherent lack of resolution in alleles as a consequence of non-neutrality. In fact, since it is evident that the larval forms of *L. viridis* and *L. ruber* are derived from the pelagic form of heteronemertean larvae, it is possible to argue that *L. viridis* and *L. ruber* demonstrate sequential grades of modification in reproductive biology that serve to minimise dispersal.

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