Genetic variation within and among infrapopulations of the marine digenetic trematode *Lecithochirium fusiforme*

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SUMMARY

Allozyme markers were used to study genetic variation in *Lecithochirium fusiforme* within a natural population of *Conger conger*. Six of 16 enzyme-coding loci studied were found to be polymorphic. These loci were surveyed in 12 infrapopulations of adult flukes. High levels of genetic variation were detected (P = 0·375); Ho = 0·048; He = 0·085). However, the population did not conform to Hardy-Weinberg expectations, as it showed a significant deficit of heterozygotes. *L. fusiforme* also exhibited low differentiation between infrapopulations (F_{ST} = 0·064). Despite significant linkage disequilibrium at *Pgm-1* and *Pgm-2* ($P < 0·05$), mating system does not appear to be the principal reason for the deficit of heterozygotes detected, because some polymorphic loci were in Hardy-Weinberg equilibrium. Association between F_{IS} and F_{ST} statistics suggests the existence of the Wahlund effect. However, all infrapopulations showed a strong deficit of heterozygotes for most polymorphic loci (F_{IS} = 0·409). Detection of significant genetic differentiation among temporal samples and the existence of paratenic hosts in the life-cycle suggests the Wahlund effect, caused by the mixture of genetically distinct temporal samples in the infrapopulations. Occasional temporal gene flow also might explain the high estimated genetic polymorphism.

Key words: allozymes, genetic variation, *Lecithochirium fusiforme*, infrapopulations.

INTRODUCTION

In the study of populations genetics a local population can be defined as 'the total sum of conspecific individuals of a particular locality comprising a single potential interbreeding unit' (Mayr, 1942). This concept of population emphasizes the idea of sexual reproduction. This requirement is crucial since the genes of the population must compose a particular pool subject to Mendelian rules that, along with the participation of different micro-evolutionary factors, will determine the genic and genotypic frequencies of the following generation. However, although effective gene flow in parasitic helminths takes place exclusively in the infrapopulations corresponding to the definitive hosts, i.e. all individuals of a species in an individual host at a particular time (Margolis *et al.* 1982), this does not mean that an infrapopulation constitutes an evolutionarily independent unit. Because the habitat within the host can be structured, it is possible that an infrapopulation contains several populations (Combes & Théron, 2000; Jarne & Théron, 2001). The infrapopulation is strongly influenced by the histories of recruitment of parasites to individual hosts, which can be very variable. It is possible, for example, that the hosts show different degrees of susceptibility to the parasitic infection, and the existence of limiting factors of the infrapopulation density, such as concomitant immunity, may cause parasitic aggregation (Nadler, 1995). Furthermore, infrapopulations are not generally considered as true populations because they constitute highly ephemeral systems that do not result from the natality in the individual host (Sire *et al.* 2001*a*).

Although genetic analysis of infrapopulations provides useful information allowing better understanding of the population structure of endoparasitic helminths, almost nothing is known about genetic structure at the infrapopulation level in marine habitats. However, some data are available on infrapopulations of adult parasitic helminths. Lydeard *et al.* (1989) detected deviations from Hardy-Weinberg expectations associated with heterozygote deficits in populations of the fluke *Fascioloides magna* in its definitive host. The deficit of heterozygotes was found to be associated with an aggregated distribution of flukes in their hosts, and with the occurrence of many specimens of the same multiple locus genotype in infrapopulations, as a result of asexual amplification in the snail host. Partly as a consequence of the parasite aggregation, populations presented weak, although significant, spatial subdivision (Mulvey *et al.* 1991). Similarly, Sire *et al.*

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(2001*a*) found significant genetic differentiation between infrapopulations of adult schistosomes at the microspatial scale, which was probably caused by a combination of limited displacement of the definitive host, spatial aggregation of infective stages and asexual multiplication in the intermediate host. In fact, the distribution of helminths, among individuals within the definitive host population is frequently aggregated (Shaw & Dobson, 1996). In *Ascaris*, significant deviations from panmixia and heterogeneity among infrapopulations were observed, possibly due to overdispersion of parasites among hosts (Anderson, Romero-Abal & Jaenike, 1995; Nadler, Lindquist & Near, 1995). In other endoparasitic helminths, subdivision at the level of the individual host was also detected (Lymbery, Thompson & Hobbs, 1990; Fisher & Viney, 1998; Paterson, Fisher & Viney, 2000). For trematodes, several identical multilocus genotypes of parasites within each individual host are more likely to occur in systems with limited cercarial dispersion, and which show an aggregated distribution of metacercariae. However, it is not known whether a similar situation exists in marine systems, where the possibilities for dispersal of the parasite are probably greater.

The hemiurid fluke *Lecithochirium fusiforme* has a life-cycle involving 3 or 4 hosts (Gibson & Bray, 1986; Matthews & Matthews, 1988). Cercariae emerge from a prosobranch mollusc, then they are ingested by copepods, and metacercaria are ingested with copepods and develop into adults in the definitive host. However, it is possible that paratenic hosts also take part in the life-cycle. The aim of this study was to describe, using allozyme markers, the amount and distribution of genetic variation within and among infrapopulations of the hemiurid fluke *L. fusiforme* in its definitive host *C. conger* and to analyse the observed pattern in relation to aspects of the life-cycle, such as the presence of the paratenic host.

MATERIALS AND METHODS

Samples and electrophoresis

A total of 806 adult *Lecithochirium fusiforme* were collected from 66 conger eels between 1998 and 2001. The specimens were subjected to enzyme electrophoresis, with the aim of obtaining an estimate of the genetic variation in the population. All fish were caught in an area of approximately 60 km², in the Ría de Arousa (a coastal embayment in Galicia north-western). The fish were eviscerated *in situ* and the digestive tracts placed in physiological saline and immediately transported to the laboratory, where they were processed. Adult flukes were collected from the stomachs of hosts and maintained alive in physiological saline for 1–2 h and then frozen at −80 °C until electrophoretic analysis. Individual specimens were homogenized using a glass rod and the homogenate was absorbed onto filter-paper wicks, which were placed into a slit cut in horizontal 10% (w/v) starch gels cooled to 4 °C. Individual host stomach tissue samples were homogenized in an equal volume of distilled deionized water using a homogenizer with blades. Host homogenates were stored for 1–3 weeks at −80 °C prior to electrophoresis. Samples of the homogenates were included in electrophoretic analysis to confirm that all isozymes of *L. fusiforme* samples were parasite-specific.

Preliminary allozyme analysis of 15 enzymes was carried out in order to estimate the degree of genetic variation in the population. Loci shown to be polymorphic were then analysed in 12 infrapopulations, selected from the 66 on the basis of their size. The enzymes tested (abbreviation and Enzyme Commission Number) were: aconitase (ACO, EC 4.2.1.3), adenosine deaminase (ADA, EC 3.5.4.4), aldolase (ALD, EC 4.1.2.13), adenilate kinase (AK, EC 2.7.4.3), diaphorase (DIA, EC 1.8.1.4), glutamate dehydrogenase (GDH, EC 1.4.1.3), glutamate oxaloacetate (GOT, EC 2.6.1.1), glucose phosphate dehydrogenase (GPD, EC 1.1.1.49), glucose phosphate isomerase (GPI, EC 5.3.1.9), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, ED 1.1.1.37), malic enzyme (ME, EC 1.1.1.40), manose phosphate isomerase (MPI, EC 5.3.1.8), nucleoside phosphorilase (NP, EC 2.4.2.1), and phosphoglucomutase (PGM, EC 2.7.5.1). All enzymes were encoded by a single locus except PGM. Thus, these enzymes provided a total of 16 scorable loci. Buffers, staining histochemical procedures and electrophoretic conditions were based on those described in Vilas *et al*. (2002) which were derived from Pasteur *et al*. (1987). Alleles were labelled alphabetically beginning with the slowest migrating electromorph in both anodal and cathodal systems.

The infrapopulations (IFP) were pooled according to the season in which they were sampled, in order to test for statistical significant differences in allele frequencies with time. The temporal samples were as follows: Winter 1997/98 (IFP1, IFP2 and IFP3); Autumn 1998 (IFP4, IFP5, IFP6, IFP7 and IFP8); Spring 1999 (IFP9 and IFP10); Winter 1999/2000 (IFP11); Autumn 2001 (IFP12).

Data analysis

Statistical analysis of electrophoretic data was carried out using GENEPOP version 3.2a (Raymond & Rousset, 1995). Genotypes and allele frequencies were determined by direct counts on the gels. Genetic variation was expressed in terms of the proportion of loci whose most common allele was not greater in frequency than 0·99 (P), as well as the observed mean heterozygosity per locus (Ho), mean

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heterozygosity per locus expected under Hardy-Weinberg equilibrium (He) and the mean number of alleles per locus (A). The numbers of homozygotes and heterozygotes expected under Hardy-Weinberg equilibrium were computed using Levene's (1949) correction for small samples. In order to investigate the extent of genetic structuring within and between *L. fusiforme* infrapopulations, Wright's *F*-statistics were used. Within each sample, deviations from Hardy-Weinberg expectations were estimated for each locus, using the F_{IS}-estimators described by Robertson & Hill (1984) and Weir & Cockerham (1984). F_{ST} and F_{IT} were also estimated as by Weir & Cockerham (1984). Fisher's exact test was used to test for deviations from Hardy-Weinberg equilibrium and for genotypic disequilibrium. Estimation without bias of exact *P*-values was made using the Markov-chain method following the algorithm of Guo & Thompson (1992). Because of the small size of the specimens, only 1 or 2 enzymes per individual were usually able to be analysed. However, linkage disequilibrium between only *Pgm-1* and *Pgm-2* was tested. Analysis of population genetic data for homogeneity between temporal samples was carried out using a Chi-square test.

Table 1. Allele frequencies in *Lecithochirium fusiforme* populations

(Exact tests for Hardy-Weinberg expectations, F_{IS} values and size sample (*N*) are shown. F_{IS} are those given by Weir & Cockerham ($F_{W\text{-}C}$) and Robertson & Hill ($F_{R\text{-}H}$).)

Locus	Allele frequencies	$F_{W\text{-}C}$	$F_{R\text{-}H}$	*P*	*N*
Aco	b = 0·044 c = 0·858 d = 0·098	0·306	0·269	< 0·01	102
Ada	c = 0·618 d = 0·382	0·649	0·654	< 0·001	114
Ak	a = 1·000	—	—	—	42
Ald	a = 1·000	—	—	—	80
Dia	a = 1·000	—	—	—	68
Gdh	a = 1·000	—	—	—	82
Got	a = 1·000	—	—	—	69
Gpd	a = 1·000	—	—	—	75
Gpi	a = 0·005 b = 0·943 d = 0·052	−0·051	−0·026	N.S.*	105
Idh	a = 0·005 b = 0·986 c = 0·010	−0·006	−0·002	N.S.	105
Mdh	a = 1·000	—	—	—	91
Me	a = 1·000	—	—	—	55
Mpi	a = 1·000	—	—	—	55
Np	a = 1·000	—	—	—	67
Pgm-1	a = 0·004 b = 0·056 c = 0·690 d = 0·077 e = 0·173	0·471	0·341	< 0·001	124
Pgm-2	a = 0·988 b = 0·012	−0·008	−0·008	N.S.	124

* N.S., Not significant.

RESULTS

The total amount of genetic variation in the population of *Lecithochirium fusiforme* in *Conger conger* from Ría de Arousa, as measured by the mean heterozygosity per locus observed (Ho = 0·048), average heterozygosity expected under Hardy-Weinberg equilibrium (He = 0·085), genetic polymorphism (P = 0·375) and mean number of alleles per locus (A = 1·75), was similar to that of populations of free living organisms (Ward, Skibinski & Woodwark, 1992). Of 16 loci studied, variation was observed in *Aco*, *Ada*, *Gpi*, *Idh*, *Pgm-1* and *Pgm-2*, within the population. The population deviated from Hardy-Weinberg expectations, for *Aco*, *Ada* and *Pgm-1* loci, which showed a significant deficit of heterozygotes (Table 1).

The 6 polymorphic loci were also analysed at the infrapopulational level, using 12 infrapopulations chosen on the basis of their size. The number of individuals studied for each locus was considerably increased therefore, as expected, new alleles were detected – in low frequencies – at *Aco*, *Ada*, *Gpi*, and *Pgm-1*. Allele frequencies in each infrapopulation, and comparisons with the values predicted from the Hardy-Weinberg equilibrium are shown in Table 2. The *Idh* and *Pgm-2* loci revealed little variation, and *Gpi* showed fixation in 1 case (IFP12). Exact tests showed a significant deviation from Hardy-Weinberg expectations for the *Pgm-1* locus in each infrapopulation, which always showed a deficit of heterozygotes. Furthermore, *Pgm-1* and *Pgm-2* revealed significant linkage disequilibrium ($P < 0·05$). There was also a significant deficit of heterozygotes for the remaining polymorphic loci. The *Ada* locus revealed deficiencies of heterozygotes in 11 infrapopulations, *Aco* in 5 and *Idh* in 2 of the 12 infrapopulations studied. By contrast, *Gpi* and *Pgm-2* did not show significant deviations from the Hardy-Weinberg equilibrium (Table 2). The $F_{W\text{-}C}$ and $F_{R\text{-}H}$ statistics provide quantification of the deviation, for each locus in each population; the values obtained were generally very high, with a total absence of heterozygotes for *Ada* in IFP1 and *Idh* in IFP12. For the 6 polymorphic loci, the most common allele in each infrapopulation was always the same, except for the *Ada* locus in infrapopulations IFP9 and IFP12 (Table 2). The population made up of all of the infrapopulations deviated from Hardy-Weinberg equilibrium, in the direction of heterozygote deficiency ($F_{IS} = 0·409$). The average fixation index F_{ST} for the 6 variable loci was 0·064 for all infrapopulations. This means that only 6·4% of the total variation can be attributed to differences among infrapopulations. The statistic $F_{IT} = 0·446$, reflected

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Table 2. Allele frequencies in *Lecithochirium fusiforme* infrapopulations (IFP)

(Exact tests for Hardy-Weinberg expectations, F_{IS} values and size sample (N) are shown. Estimators of F_{IS} are those given by Weir & Cockerham ($F_{W\text{-}C}$) and Robertson & Hill ($F_{R\text{-}H}$).)

	IFP1	IFP2	IFP3	IFP4	IFP5	IFP6	IFP7	IFP8	IFP9	IFP10	IFP11	IFP12
Aco												
a	0·000	0·000	0·000	0·000	0·000	0·010	0·000	0·000	0·000	0·000	0·000	0·017
b	0·013	0·054	0·069	0·020	0·033	0·020	0·106	0·060	0·094	0·037	0·016	0·000
c	0·833	0·838	0·845	0·961	0·783	0·949	0·712	0·767	0·823	0·737	0·944	0·881
d	0·154	0·108	0·086	0·020	0·167	0·020	0·182	0·172	0·083	0·225	0·024	0·000
e	0·000	0·000	0·000	0·000	0·017	0·000	0·000	0·000	0·000	0·000	0·016	0·102
N	39	37	29	51	30	49	33	58	48	40	63	59
P	<0·001	N.S.*	N.S.	N.S.	<0·01	N.S.	N.S.	<0·001	N.S.	<0·001	<0·05	N.S.
$F_{W\text{-}C}$	0·911	0·250	−0·115	0·237	0·454	−0·026	0·070	0·414	0·128	0·514	0·265	0·211
$F_{R\text{-}H}$	0·512	0·202	−0·076	0·000	0·185	−0·008	0·044	0·475	0·053	0·572	0·330	0·128
Ada												
a	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·006	0·000	0·008	0·000
b	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·008	0·000
c	0·816	0·881	0·742	0·670	0·600	0·660	0·740	0·594	0·360	0·808	0·614	0·403
d	0·184	0·119	0·258	0·330	0·400	0·340	0·260	0·406	0·634	0·192	0·371	0·597
N	38	42	33	50	30	50	25	53	86	39	132	67
P	<0·001	<0·01	<0·001	<0·001	N.S.	<0·001	<0·05	<0·001	<0·001	<0·001	<0·001	<0·01
$F_{W\text{-}C}$	1·000	0·554	0·769	0·510	0·183	0·649	0·496	0·731	0·582	0·758	0·566	0·386
$F_{R\text{-}H}$	1·000	0·565	0·790	0·518	0·187	0·660	0·511	0·743	0·301	0·775	0·199	0·390
Gpi												
a	0·000	0·010	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·012	0·021	0·000
b	0·927	0·898	0·964	0·950	0·915	0·860	0·900	0·970	0·990	0·951	0·889	1·000
c	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·010	0·000	0·000	0·000	0·000
d	0·049	0·092	0·036	0·050	0·085	0·140	0·100	0·020	0·010	0·037	0·090	0·000
e	0·024	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000
N	41	49	69	50	41	50	40	50	52	41	72	46
P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	—	N.S.	N.S.	—
$F_{W\text{-}C}$	−0·048	−0·092	−0·030	−0·043	0·231	−0·153	0·179	−0·014	0·000	−0·029	0·041	—
$F_{R\text{-}H}$	−0·027	−0·047	−0·030	−0·043	0·234	−0·154	0·182	−0·005	0·000	−0·013	0·029	—
Idh												
a	0·000	0·020	0·000	0·000	0·000	0·010	0·025	0·000	0·000	0·000	0·000	0·022
b	1·000	0·959	0·986	0·990	0·988	0·960	0·962	0·990	1·000	0·988	0·993	0·957
c	0·000	0·020	0·014	0·010	0·012	0·030	0·013	0·010	0·000	0·012	0·007	0·022
N	41	49	69	50	41	50	40	50	52	41	72	46
P	—	<0·05	N.S.	—	—	N.S.	N.S.	—	—	—	—	<0·001
$F_{W\text{-}C}$	—	0·492	−0·007	0·000	0·000	−0·023	−0·017	0·000	—	0·000	0·000	1·000
$F_{R\text{-}H}$	—	0·505	−0·007	0·000	0·000	−0·011	−0·007	0·000	—	0·000	0·000	1·000
Pgm-1												
a	0·000	0·000	0·000	0·000	0·000	0·042	0·000	0·009	0·008	0·000	0·009	0·000
b	0·081	0·048	0·051	0·043	0·035	0·217	0·036	0·045	0·000	0·036	0·079	0·027
c	0·512	0·731	0·551	0·734	0·628	0·450	0·718	0·655	0·805	0·595	0·754	0·857
d	0·035	0·038	0·013	0·021	0·023	0·067	0·018	0·173	0·161	0·214	0·035	0·080
e	0·360	0·183	0·385	0·191	0·314	0·225	0·227	0·118	0·025	0·155	0·123	0·036
f	0·012	0·000	0·000	0·011	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000
N	43	52	39	47	43	60	55	55	59	42	57	56
P	<0·05	<0·05	<0·01	<0·05	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·01	<0·001
$F_{W\text{-}C}$	0·313	0·247	0·400	0·254	0·548	0·335	0·626	0·557	0·537	0·716	0·278	0·657
$F_{R\text{-}H}$	0·247	0·348	0·159	0·055	0·524	0·377	0·558	0·346	0·196	0·531	0·152	0·612
Pgm-2												
a	0·942	1·000	0·987	1·000	0·977	0·925	1·000	1·000	0·992	1·000	0·982	1·000
b	0·058	0·000	0·013	0·000	0·023	0·075	0·000	0·000	0·008	0·000	0·018	0·000
N	43	52	39	47	43	60	55	55	59	42	57	56
P	N.S.	—	—	—	N.S.	N.S.	—	—	—	—	N.S.	—
$F_{W\text{-}C}$	−0·050	—	0·000	—	−0·012	0·167	—	—	0·000	—	−0·009	—
$F_{R\text{-}H}$	−0·051	—	0·000	—	−0·012	0·169	—	—	0·000	—	−0·009	—

* N.S., Not significant.

the reduction in heterozygosity due to nonrandom mating within infrapopulations (F_{IS}) plus that due to population subdivision (F_{ST}) (Table 3). When the infrapopulations were pooled according to the season in which they were sampled, significant differences in allele frequencies were detected for the *Aco*, *Ada*, *Gpi* and *Pgm-1* loci among such temporal groups (Table 4).

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Table 3. *F*-statistics for *Lecithochirium fusiforme* infrapopulations

	Aco	*Ada*	*Gpi*	*Idh*	*Pgm-1*	*Pgm-2*	All
F_{IS}	0·310	0·578	−0·001	0·263	0·445	0·059	0·409
F_{ST}	0·035	0·099	0·021	0·000	0·059	0·031	0·064
F_{IT}	0·335	0·620	0·020	0·263	0·478	0·088	0·446

DISCUSSION

The estimated allozyme variability in the population studied was relatively high and similar to that in other populations of digeneans (Lydeard *et al*. 1989; Dybdahl & Lively, 1996). However, in contrast with the latter study, where there was no indication of either inbreeding or further subdivision of populations, the genotypic frequencies of the population studied did not conform to Hardy-Weinberg equilibrium predictions. This deviation was always towards a deficit of heterozygotes. There are several possible causes of heterozygote deficits in natural populations, such as natural selection, nonrandom mating, the Wahlund effect or the presence of null alleles. When several populations or demes with different allele frequencies are pooled and considered a single a panmictic unit, deficiencies in the number of heterozygous genotypes may be observed, even though Hardy-Weinberg proportions may hold true within the subgroups; this reduction in heterozygotes is caused by Wahlund's principle. High frequencies of null phenotypes for isozymes have been reported for a sympatric population of another species of the genus *Lecithochirium* (Vilas *et al*. 2001). However, the presence of null alleles is less likely here, because null phenotypes were not detected. Selection against heterozygotes is also unlikely because deviations were detected for 3 of the 6 polymorphic loci analysed.

The most common cause for deficit of heterozygotes in populations of trematodes probably lies in the reproductive pattern. The presence of asexual multiplication in the snail host, the possibility of self-crossing, mating between individuals with the same genotype (resulting from clonal reproduction) in the definitive host, and habitat patchiness represented by the host population are all factors that can generate an enormous degree of inbreeding in the parasite populations, and therefore may account for serious deficits of heterozygotes. Under a model of this type, where other causes for deviation of Hardy-Weinberg expectations are less important, the F_{IS} fixation index should be similar for different loci, but this was not the case in the present study. Furthermore, *Gpi* and *Pgm-2* were in Hardy-Weinberg equilibrium in each infrapopulation and in the population when was considered as the combination of infrapopulations. Thus, the reproductive system does not appear to be the main reason for the heterozygote deficit detected. However, because all F_{IS} values obtained in the infrapopulations were very high when compared to reported values for other parasites (Mulvey *et al*. 1991; Nadler *et al*. 1995), this result is not consistent with random recruitment from a larger panmictic pool of parasite life-cycle stages.

The lack of correspondence in the population, with Hardy-Weinberg predictions may also to be explained by the existence of interdemic genetic variance (the Wahlund effect). A strong association between F_{IS} and F_{ST} was detected. This result, i.e. that the loci showing the greatest deviation from Hardy-Weinberg equilibrium are those that revealed the greatest degree of differentiation among infrapopulations, suggests the presence of the Wahlund effect. However, the heterozygote deficit was not simply a consequence of the consideration of several infrapopulations with significantly different allele frequencies as a panmictic unit, because each infrapopulation revealed deficiencies of heterozygotes at several polymorphic loci. The deviation detected was therefore the result of the mixture of distinct infrapopulations whose allele frequencies did not correspond to Hardy-Weinberg expectations. However, deviation within each infrapopulation may be caused by the Wahlund effect. It is possible that infrapopulations are structured as a result of habitat heterogeneity (Combes & Théron, 2000). However, this is unlikely here because we observed a random physical distribution pattern of the parasite in the stomach. It is also possible that infrapopulations in the definitive hosts contain a mixture of genetically different population samples, a situation that is favoured by mobility of the host. Although the mobility of conger eels is limited in the study area, most of the genetic variation within the population was explained by variation within each infrapopulation rather than variation among them. Therefore, no genetic structure was revealed in the population studied. It is also possible that different intermediate or paratenic hosts serve as selective filters that shape different populations that co-exist in the definitive host (George-Nascimento & Llanos, 1995). The effect of natural selection would be reinforced if asexual reproduction was predominant in the population. Although heterozygote deficiencies do not appear to be caused by mating system alone, the finding of significant linkage disequilibrium at *Pgm-1* and *Pgm-2* suggests that the homogenizing effect of migration and sexual reproduction in the population, are not sufficient to cause the complete disintegration of certain clonal structure resulting from the asexual reproduction of the parasite within snail hosts. A putative role of the natural selection in the genesis of the Wahlund effect in the definitive host may be related to a certain degree of versatility in the life-cycle of *Lecithochirium* species, depending on the presence or absence of paratenic hosts.

Table 4. Allele frequencies in temporal samples of *Lecithochirium fusiforme* population

(Sample size (*N*) and homogeneity Chi-square are shown.)

Locus	Winter 1997/98	Autumn 1998	Spring 1999	Winter 1999/2000	Autumn 2001	χ^2
Aco						
a	0·000	0·002	0·000	0·000	0·017	18·68*
b	0·043	0·045	0·068	0·016	0·000	
c	0·838	0·846	0·784	0·944	0·881	
d	0·119	0·104	0·148	0·024	0·102	
e	0·000	0·002	0·000	0·016	0·000	
N	105	221	88	63	59	
Ada						
a	0·000	0·000	0·004	0·008	0·000	81·77***
b	0·000	0·000	0·000	0·008	0·000	
c	0·819	0·647	0·500	0·614	0·403	
d	0·181	0·353	0·496	0·371	0·597	
N	113	208	125	132	67	
Gpi						
a	0·003	0·000	0·005	0·021	0·000	17·37**
b	0·934	0·920	0·973	0·889	1·000	
c	0·000	0·002	0·000	0·000	0·000	
d	0·057	0·078	0·021	0·090	0·000	
e	0·006	0·000	0·000	0·000	0·000	
N	159	231	93	72	46	
Idh						
a	0·006	0·006	0·000	0·000	0·022	6·39 N.S.
b	0·981	0·978	0·995	0·993	0·957	
c	0·013	0·015	0·005	0·007	0·022	
N	159	231	93	72	46	
Pgm-1						
a	0·000	0·012	0·005	0·009	0·000	71·24***
b	0·060	0·081	0·015	0·079	0·027	
c	0·608	0·631	0·718	0·754	0·857	
d	0·030	0·063	0·183	0·035	0·080	
e	0·298	0·211	0·079	0·123	0·036	
f	0·004	0·002	0·000	0·000	0·000	
N	134	260	101	57	56	
Pgm-2						
a	0·978	0·979	0·995	0·982	1·000	4·97 N.S.
b	0·022	0·021	0·005	0·018	0·000	
N	134	260	101	57	56	

* $P<0·05$; ** $P<0·01$; *** $P<0·001$; N.S., not significant.

The conger eel is a teleostean with very diverse food sources, it mainly preys on other fishes (Cau & Manconi, 1984). This eel sometimes ingests *L. fusiforme* by feeding on small fishes such as gobiids and labrids, which harbour encapsulated immature specimens of the parasite (Gibson & Bray, 1986). Detection of a high prevalence of immature *Lecithochirium* in these fishes appears to indicate that such paratenic hosts play a important role in the life-cycle of the parasite (Santos & Eiras, 1995; Abollo, Gestal & Pascual, 1998). The existence of paratenic hosts may influence the genetic composition of the infrapopulations in the definitive host in different ways. The levels of genetic variation in infrapopulations of adult trematodes depend on different factors, including genotypic overdispersion within intermediate hosts, effective population size, and mobility and behaviour of the vertebrate host (Minchella, Sollenberger & Pereira De Souza, 1995; Barral *et al.* 1996; Sire *et al.* 2001*a*, *b*). Despite restricted displacement of snails and copepods which integrate populations structured highly (Burton & Feldman, 1981; Jarne & Théron, 2001), the fact that in the study area the conger eels are present in a sedentary stage of their life-cycle (Cau & Manconi, 1983), and show territoriality, the mobility of infected small fishes that act as hosts to *L. fusiforme* may cause low genetic differentiation between infrapopulations and high genetic polymorphism in the parasite population. In the population studied F_{ST} values were low, indicating little genetic differentiation among infrapopulations at the microgeographical level. Apart from host mobility, the high prevalence and intensity of infection of this worm in *C. conger* in the

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Ría de Arousa (Paniagua & Vilas, 2001), may also explain the high levels of genetic variation and low F_{ST} values observed.

The presence of paratenic hosts in the life-cycle of *L. fusiforme* may also influence the genetic structure of parasite populations, not only by introducing new selective pressures, but also by determining the level of interdemic gene flow. Despite low genetic differentiation among infrapopulations and a high mean intensity of parasite infection, genetic drift may exert an important effect on the population. There are several reasons that suggest strong genetic drift in populations of digeneans. These populations probably undergo greater demographic fluctuations and the genetic composition of infrapopulations may change significantly in each generation by chance, because of high mortalities and high reproductive rates. Although fluke populations appear to have large population census size, the effective size of the population must be small because the distribution of flukes is very patchy, the infrapopulations are very ephemeral systems, and it is probable that each of them consists of an important amount of genetically identical individuals, as a result of asexual amplification. Although the presence of persistent life-cycle stages and mobility of paratenic hosts are factors that reduce genetic structure in the population and, accordingly, they may explain the low F_{ST} values obtained (Nadler, 1995), it is also possible that random change in allele frequencies cause important genetic divergence over time in this type of population. In the present study, when infrapopulations were pooled as temporal samples, significant differences in allele frequencies were detected. Although it is very likely temporal gene flow because of frequent ingestion by the conger eels of fishes which harbour persistent life-cycle stages of *L. fusiforme*, such gene flow may not be sufficient to avoid some significant genetic differentiation in the time caused by genetic drift. The mixture of different temporal samples, with significantly different allele frequencies by genetic drift, may cause the Wahlund effect and may therefore account for the deviation of the Hardy-Weinberg equilibrium within infrapopulations. Because this mixture represents an important source of genetic variation in the population, occasional temporal gene flow may also explain the high degree of genetic polymorphism estimated.

No doubt, levels of genetic variation within and among infrapopulations in a particular location are the result of different factors, some of which have opposite effects. Knowledge of the genetic structure of natural populations of marine digenetic trematodes is clearly insufficient. Besides studies of genetic variation at the infrapopulation level, studies are necessary that involve analysis of the distribution of the genetic variability including a certain geographical range, as well as variation in the time within a same locality. These studies would allow clarification of whether infrapopulations actually integrate different populations, and the degree to which this hypothesis can be generalized to other marine endoparasitic helminths with complex life-cycles.

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