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Experimental approach to the importance of parasitism in biological conservation

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ABSTRACT: Recolonisation of protected areas by new host species, and their parasites, or the translocation of individuals (accidentally or intentionally) to new locations may induce new host-parasite associations. Parasites are usually found to be less well-adapted and more virulent to newly colonized host species. Such new host-parasite associations may represent threats to the survival of the host populations. Our study compared the reaction of a naive host facing a new parasite with the reaction of a host in a population already associated with that parasite. We collected 305 *Pomatoschistus microps* (Krøyer, 1838), second intermediate host of the digenean *Labratrema minimus* (Stossich, 1887), from 4 regions around Europe. The fish were experimentally exposed to the parasite strain endemic to one of these regions. The initial step consisted of evaluating the genetic variation among the different fish populations. The genetic results, based on isozyme electrophoresis, revealed a significant differentiation among the populations studied. The second step determined both the quantitative and qualitative success of infestation of the different host populations. Our results show that there is no quantitative difference between sympatric host-parasite success and allopatric success. However, at the ultrastructural level, sympatric infection appears to be more successful. The results are discussed in terms of the local adaptation of host-parasite associations and the consequences to biological conservation.

KEY WORDS: Fish · Parasite · Local adaptation · Biological conservation · Translocation · Digenean · *Labratrema minimus* · *Pomatoschistus microps*

INTRODUCTION

Conserving biological diversity has become one of the most important ecological goals over the last 20 yr. Numerous protected areas have been created to protect *in situ* biological diversity at both the species and gene level. Although too few in number and of limited overall area (Moyle & Leidy 1992), protected areas have 3 main functions: (1) to maintain species-rich areas free of all exploitation; (2) to maintain sufficient individuals of an endangered species to ensure a viable population capable of recolonizing surrounding, unprotected zones; (3) to enable overexploited or displaced species to recolonize their habitat (Harmelin 1987, Francour 1994, Dufour et al. 1995).

In addition to the creation of protected areas, there are other methods of conserving diversity that involve the transfer of individuals from regions with viable populations to regions where that species is rare or absent (Konstant & Mittermeier 1982, Minckley 1995). Generally, such manipulations are now much less frequently considered or very carefully implemented. Intentional transfer of species is only carried out when the genetic history of the introduced individuals, the reasons for the disappearance of the earlier population, the field conditions, and the densities of interacting species (i.e. any local population that may still be present, predators and prey) are very well known (Kleiman 1989, Lindburg 1992, Lodge et al. 1998). During transfer of protected species or of species for aquaculture, one of the biggest problems is to anticipate the possible effects of parasites accidentally introduced with the transfer of the host species (Calvo-Ugarteburu

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& McQuaid 1998). Parasites may affect their host differently in a different environment; they may also colonize new hosts among the resident indigenous species. Several examples of unwanted introduction of parasites and their dramatic effects on natural populations have been reported (Bauer & Hoffman 1976, Dwyer et al. 1990, Viggers et al. 1993). Moreover, it has been shown that such a new parasitic relationship can evolve rapidly and nullify the success of the introduction (Dwyer et al. 1990, Carroll & Boyd 1992).

The capacity of a parasite to successfully infect a host is mainly based on its ability to avoid host defense-mechanisms. Intentional or accidental transfer of a host species would presumably alter the epidemiology of a parasitic relationship because of the changed environmental conditions. Modeling studies have shown parasite infectivity to be closely related to epidemiological factors (Claessen & de Roos 1995), and that sympatric hosts and parasites should be more compatible than allopatric ones (Hamilton 1993, Frank 1994, Gandon et al. 1996, Morand et al. 1996). However, few studies have empirically demonstrated the greater compatibility of local associations compared to non-native associations (Vera et al. 1990, Ballabeni & Ward 1993, Ebert 1994, Lively & Jokela 1996). Moreover, it generally appears, in the case of invertebrates or vertebrates infected with macroparasites, that local adaptation is not ubiquitous (Soler & Møller 1990, Briskie et al. 1992, Ballabeni & Ward 1993, Grosholz & Ruiz 1995, Dufva 1996, Lively & Jokela 1996). Empirical studies have shown that in several cases a parasite may evolve through a minimal virulence value (Toft & Karter 1990, Ewald 1995), resulting in optimal success of the host-parasite association.

The evolution of host-parasite systems has mostly been ignored in biological conservation (Scott & Dobson 1989, McCallum & Dobson 1995). Several cases of simultaneous host and parasite introduction have been reported for aquatic ecosystems (Hoffman 1970, Combes & Le Brun 1990, Moravec 1992, Kennedy 1993, 1994). Host translocation may lead to 4 types of new host-parasite associations: (1) the establishment of a new host-parasite system in the area; (2) the establishment of a new parasite species in a zone where the local host population was previously free of the parasite (for example, because of the lack of an intermediate host in the case of indirect life-cycle parasite species or because of a locally resistant host population); (3) the establishment of a non-infected host population into a zone inhabited by the local population are infested by a parasite species; (4) the introduction of a host species into a zone inhabited by a generalist parasite species (a species able to complete its life cycle on several host species) to which it is vulnerable.

This paper aimed at determining if there is a differential reaction among several populations of a single fish species exposed to a single strain of a parasite species. The initial step determined patterns of genetic variation among populations of the host; the second step evaluated the host/parasite association of the different fish populations both quantitatively (epidemiologically) and qualitatively (histologically) at each sampling location.

MATERIALS AND METHODS

The host-parasite system. We examined the digenean bucephaliid *Labratrema minimus* (Stossich, 1887) and one of its second intermediate hosts, the gobiid fish *Pomatoschistus microps* (Krøyer, 1838). Maillard (1976) has reported the parasite's life cycle: The first intermediate host is the lamellibranch mollusc *Cerastoderma glaucum* (Bruguiere, 1789), and the final host is the sea bass *Dicentrarchus labrax* (Linnaeus, 1758). The eggs of the parasite are disseminated through the faeces of the final host. Miracidia are liberated, and infect the mollusc through skin penetration. Asexual reproduction occurs in the snail, and liberated cercariae actively penetrate the second intermediate host. These cercariae encyst as a metacercaria in the liver of this second intermediate host. The final host becomes infested by eating the second intermediate host.

Host samples. We collected more than 100 first intermediate hosts, *Cerastoderma glaucum*, in Salses-Leucate lagoon (Southern France 42° 50' N, 03° 00' E), but only 3 of these shed cercariae. We can, therefore, assume limited genetic variability in the pool of infecting parasites in our experiments.

A total of 296 gobiids *Pomatoschistus microps* were captured live, with a seine net, at 4 different stations around Europe, during spring 1996 (Fig. 1). The fish were obtained from the (1) Salses-Leucate lagoon (Mediterranean Sea, Southern France: 42° 50' N, 03° 00' E, n = 70); (2) near Cadiz in the Guadalquivir river estuary (Atlantic Ocean, southern Spain: 36° 30' N, 06° 20' W, n = 100); (3) Hendaye in the Chingudy Bay (Atlantic Ocean, south-west France: 43° 30' N, 01° 50' W, n = 73) and (4) the island of Fehmarn (North Sea, northern Germany: 50° 30' N, 11° 10' E, n = 53).

Electrophoresis and genetic analysis. Skeletal muscle was dissected from fish, disrupted in 1 ml 0.2 M Tris-HCl buffer, and centrifuged for 10 min at 4000 × *g* and 4°C. The supernatant protein extract was then frozen and kept at –80°C until required. Proteins were separated by horizontal starch gel electrophoresis according to Pasteur et al. (1987). Loci and alleles were designated according to Shaklee et al. (1990). The

Table 1. *Pomatoschistus microps*. Protein systems examined, their loci and allelic variability. All systems were evaluated using skeletal muscle, and buffer systems were adapted according to Wallis & Beardmore (1984) or Pasteur et al. (1987). TBE: tris-borate, EDTA; TC: tris citrate; TCE: tris-citrate EDTA

Protein system	Locus	Variability	Buffer
Adenosine deaminase (EC 3. 5. 4. 4)	ADA	Polymorphic	TC 8.0
Adenylate kinase (EC 2. 7. 4. 3)	AK	Monomorphic	TC 8.0
Aspartate amino transferase (EC 2. 6. 1. 1)	AAT	Polymorphic	TBE 8.0
Creatine kinase (EC 2. 7. 3. 2)	CK1	Monomorphic	TC 8.0
	CK2	Monomorphic	TC 8.0
Fumarase (EC 4. 2. 1. 2)	FUM	Polymorphic	TC 8.0
Guanine deaminase (EC 3. 5. 4. 3)	GDA	Monomorphic	TC 8.0
Isocitrate dehydrogenase (EC 1. 1. 1. 42)	IDH	Polymorphic	TC 8.0
Lactate dehydrogenase (EC 1. 1. 1. 27)	LDH1	Monomorphic	TCE 8.7
	LDH2	Monomorphic	TCE 8.7
Malate dehydrogenase (EC 1. 1. 1. 37)	MDH1	Polymorphic	TC 8.0
	MDH2	Polymorphic	TC 8.0
Mannose phosphate isomerase (EC 5. 3. 1. 8)	MPI	Polymorphic	TCE 8.7
Peptidase leucyl glycyl glycine (EC 3. 4. 11-)	PEP B	Polymorphic	TC 8.0
Peptidase phenylalanyl leucine (EC 3. 4. 11-)	PEP E	Polymorphic	TC 8.0
Phosphogluconate deshydrogenase (EC 1. 1. 1. 43)	PGD	Polymorphic	TCE 8.7
Phosphoglucoisomerase (EC 5. 3. 1. 9)	PGI1	Polymorphic	TCE 8.7
	PGI2	Polymorphic	TCE 8.7
Phosphoglucomutase (EC 2. 7. 5. 1)	PGM1	Polymorphic	TC 8.0
	PGM2	Polymorphic	TC 8.0

most commonly observed allele in Salses-Leucate (the sympatric population) was assigned a mobility of 100. Fifteen enzymes encoded by 20 putative loci were examined using 3 different buffer systems (Table 1). Within-sample variability was assessed by calculating the percentage of polymorphic loci at 95% level P_{95}, mean number of alleles per locus (A), mean observed (H_o) and unbiased expected (H_e) heterozygosity per locus. Measures of allozyme variability were estimated using GENETIX v3.0 (Belkhir et al. 1996), and F-statistics estimators were calculated using GENEPOP v3.1 (Raymond & Rousset 1995). Genetic distances between populations were calculated as D = -ln (1-theta) (Reynolds et al. 1983), and were displayed by UPGMA (unweighted pair-group method using arithmetic averages) (Sneath & Sokal 1973).

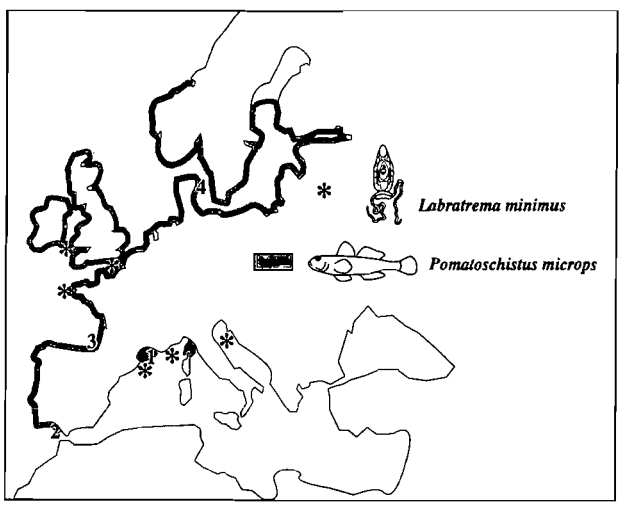

Fig. 1. Spatial distribution in western Europe of *Pomatoschistus microps* (according to Whitehead et al. 1986) and *Labratrema minimus* (according to Maillard 1976). 1: Salses-Leucate; 2: Cadiz; 3: Hendaye; 4: Fehmarn

Experimental infestations. Before the experiments, salinity was progressively adjusted to 28‰, corresponding to the salinity at which the molluscs shed the digenean cercariae. Fish were kept in an aerated aquarium in a climate-controlled room (20°C), and were fed every other day with frozen brine shrimps (*Artemia* sp.). As the parasite species is reported to be ubiquitous along the coasts of Europe, we evaluated the initial level of infection of each population by counting encysted metacercaria in fish from each collection region prior to experimental infestation (8, 10, 12 and 13 fishes were used for Fehmarn, Cadiz, Salses-Leucate and Hendaye, respectively). From each region, 30 fish (26 for Fehmarn) were experimentally infested. Individual fish were placed with 30 cercariae in 100 ml seawater for 1 h. Fish were necropsied 1 mo later. Livers with encysted metacercariae were either dissected to quantitatively evaluate the infection success, or fixed in Bouin's solution (Martoja & Martoja 1967) to evaluate the qualitative success of infection at the histological level.

Before dissection, the liver was immersed for 1 night in a 0.25% solution of potassium hydroxide (KOH). The following morning, the dissolved livers were fil-

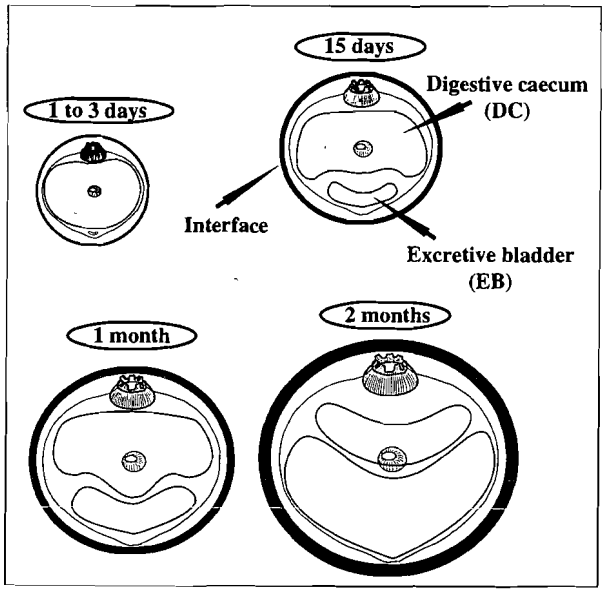

Fig. 2. *Labratrema minimus*. Schematic representation of metacercariae during first 2 mo of development (after Faliex 1990)

tered on a 40 µm filter, and encysted metacercariae were stained with Lugol's solution and counted under a binocular microscope. We estimated infestation success for each individual (S_{ind}) and for the population (taking into account the fish that died during the experiment) (I_{pop}):

$$S_{ind} = \frac{N_{+30} - N_0}{30} \times 100$$

and

$$I_{pop} = \frac{\sum N_{+30} - N_0}{N_{HI} \times 30} \times 100$$

where N_{+30} = total number of encysted metacercariae after infestation with 30 cercariae; N_0 = mean initial number of metacercariae in the considered population; N_{HI} = initial number of infected hosts.

For the histological study, livers were sectioned (7 µm width) and stained according to the method of Martoja & Martoja (1967). Histological evaluation of the infestation success for each population was limited to measuring the metacercarial development 1 mo after infestation. Thus, we measured the volume of the digestive caecum and of the excretive bladder to evaluate metacercarial metabolic activity (Fig. 2). We also measured the volume of the metacercaria, the volume of the metacercarial cyst, and the width of the interface zone between the cyst and the fish's hepatic cells (Fig. 2). All measurements were made with an ocular micrometer, and volumes were estimated for a regular ovoid the formula $V = (\pi \times \text{length} \times \text{width}^2)/6$.

We used non-parametric Mann-Whitney *U*-tests within one geographical zone or to compare 2 stations, and multiple-comparison Tukey-type tests for comparison among all 4 stations (Zar 1984). Discriminant analysis was performed on the histological measurements of the metacercarial cyst to calculate the Mahalanobis distances. A cluster-analysis similarity tree was performed on the Mahalanobis distances using the UPGMA method. A Mantel test between genetic and Mahalanobis distances was performed with 1000 permutations (Mantel 1967).

RESULTS

Initial comparisons

Size and condition of *Pomatoschistus microps* differed significantly among the 4 regions. Fish from Hendaye were largest (Mann-Whitney *U*-test, $p < 0.001$) (Table 2). The Salses-Leucate fish were significantly larger than those at the 2 remaining stations (Mann-Whitney *U*-test, $p < 0.001$) (Table 2). Hepatosomatic indexes did not differ significantly between sampling areas (Mann-Whitney *U*-test, $p > 0.05$) (Table 2). Condition factors at Salses-Leucate were significantly lower than at Cadiz and Fehmarn (Mann-Whitney *U*-test, $p < 0.001$) (Table 2).

Within each region, fish samples taken to assess natural levels of infestation did not differ in size from the experimental fish (Mann-Whitney *U*-test, $p > 0.05$ for all stations). Hepatosomatic index and condition factors were not compared before and after the experiment, because during the month-long experiment food was provided at libidum. This would result in improved condition.

Table 2. *Pomatoschistus microps*. Comparison of the biological factors between stations. SL: standard length; HSI: hepatosomatic index; K: condition factor (all factors ± SE)

Area	SL (cm)	HSI (%)	K (10^{-3} g cm^{-3})
Salses-Leucate (1)	3.50 ± 0.03	3.54 ± 0.35	9.8 ± 0.2
Cadiz (2)	3.18 ± 0.03	3.69 ± 0.16	10.8 ± 0.2
Hendaye (3)	3.77 ± 0.04	3.34 ± 0.15	10.6 ± 0.2
Fehmarn (4)	3.15 ± 0.04	4.44 ± 0.38	11.3 ± 0.2
Mann-Whitney	1 > 2; p < 0.001 1 > 4; p < 0.001 3 > 1; p < 0.01 3 > 2; p < 0.001 3 > 4; p < 0.001	All comparisons NS	2 > 1; p = 0.01 4 > 1; p < 0.001 Other comparisons NS

Fish from the Salses-Leucate lagoon (sympatric association) were significantly more naturally infested than those from the other stations (multiple-comparison Tukey-type test, $p < 0.01$ for all stations). Fish from Cadiz were not naturally infected, even though *Labratrema minimus* is reported to be ubiquitous along the coasts of Europe (Maillard 1976).

Genetic comparisons

Genetic analysis revealed 14 scorable polymorphic loci (Table 3). Two loci (FUM and MDH1) were diagnostic between fish collected from the Mediterranean Sea (Salses-Leucate) and those from the other sampling areas. Locus MDH2 was not polymorphic (allele frequency <5%). The rate of polymorphism (P_{95}) among the remaining loci was high, ranging from 0.263 in Hendaye and Fehmarn to 0.421 in Cadiz (Table 4). Mean expected heterozygosity (H_e) ranged from 0.119 in Fehmarn to 0.179 in Cadiz, while the mean number of alleles per locus ranged from 1.53 to 2.16 in Fehmarn and Cadiz, respectively (Table 4).

There was a significant deviation from Hardy-Weinberg expectation for several loci within samples (positive F_{IS} see Table 5). Chi-square tests for homogeneity of allele frequencies over 10 polymorphic loci revealed significant differences at ADA, IDH, MPI, PEP2, PGI2 and PGM1 (Table 5). High genetic divergence was found between Salses-Leucate and all other samples ($F_{ST} = 0.635$ for Cadiz, 0.657 for Hendaye, and 0.660 for Fehmarn). Inter-sample genetic differentiation (F_{ST}) between Cadiz, Hendaye and Fehmarn displayed lower values (F_{ST} Cadiz/Hendaye, 0.061; Cadiz/Fehmarn, 0.142; Hendaye/Fehmarn, 0.110).

Quantitative experimental infestation success

The individual infestation success (S_{ind}) was higher for Salses-Leucate and Hendaye (multiple-comparison Tukey-type test, $p < 0.05$) (Table 6). When the number of dead fish (i. e. number of dead individuals due to the parasite infestation) was taken into account, success (I_{pop}) was significantly higher in Cadiz and lower in Fehmarn (multiple-comparison Tukey-type test, $p < 0.05$) (Table 6).

Qualitative experimental infestation success

The volume of the metacercaria, the volume of the digestive coecum, and the width of the interface were significantly greater in the sympatric association (Salses-Leucate) than in the allopatric

Table 3. *Pomatoschistus microps*. Allele frequencies at 14 polymorphic loci in samples from 4 areas. –: absent

Locus	Allele	Salses-Leucate (n = 57)	Cadiz (n = 61)	Hendaye (n = 69)	Fehmarn (n = 50)
AAT	79	–	1.000	1.000	1.000
	89	0.053	–	–	–
	100	0.947	–	–	–
ADA	63	0.070	–	–	–
	75	0.044	–	–	–
	88	0.219	0.008	0.029	–
	95	–	–	–	–
	100	0.272	0.025	0.217	–
	107	0.009	–	–	–
	112	0.263	0.057	0.022	–
	122	0.123	0.385	0.275	0.580
	140	–	0.525	0.457	0.420
FUM	92	–	1.000	1.000	1.000
	100	1.000	–	–	–
IDH	88	0.018	0.270	0.210	–
	100	0.982	0.443	0.681	1.000
	108	–	0.287	0.109	–
MDH-1	82	–	1.000	1.000	1.000
	100	1.000	–	–	–
MDH-2	47	0.018	1.000	1.000	1.000
	100	0.982	–	–	–
MPI	85	–	0.033	–	0.030
	90	0.026	0.328	0.159	0.100
	95	0.079	0.451	0.348	0.430
	100	0.895	0.188	0.493	0.440
PEP B	89	–	–	0.007	0.010
	100	1.000	0.902	0.993	0.990
	116	–	0.098	–	–
PEP E	65	–	0.008	–	–
	88	0.053	0.008	–	–
	100	0.912	0.976	1.000	1.000
	112	0.035	0.008	–	–
PGD	100	0.886	1.000	1.000	1.000
	107	0.114	–	–	–
PGI-1	85	–	0.606	0.580	0.490
	93	0.166	0.369	0.420	0.510
	100	0.816	0.025	–	–
	107	0.009	–	–	–
	115	0.009	–	–	–
PGI-2	57	–	0.271	0.065	0.060
	100	0.991	0.729	0.935	0.940
	143	0.009	–	–	–
	157	–	–	–	–
PGM-1	91	0.105	–	–	–
	100	0.860	–	–	–
	112	0.035	0.148	0.015	–
	115	–	0.844	0.985	0.600
	126	–	0.008	–	0.400
PGM-2	58	–	–	–	0.010
	88	0.026	0.025	0.015	–
	100	0.719	0.942	0.970	0.990
	112	–	0.008	–	–
	117	0.255	0.025	0.015	–

Table 4. *Pomatoschistus microps*. Measures of allozyme variability in samples from the different sampling areas. P_{95}: percentage of polymorphic loci at 95%; *A*: mean number of allele per locus. H_o: observed heterozygosity. H_e: expected unbiased heterozygosity under Hardy-Weinberg equilibrium

Area	Sample size	P_{95}	*A*	H_o	H_e
Salses-Leucate	57	0.400	2.15	0.104	0.131
Cadiz	61	0.421	2.16	0.165	0.179
Hendaye	69	0.263	1.84	0.114	0.134
Fehmarn	50	0.263	1.53	0.093	0.119

associations (multiple-comparison Tukey-type test, $p < 0.01$) (Table 6). Moreover, the volume of the cyst cavity and the volume of the excretory bladder were significantly larger for Salses-Leucate and Fehmarn samples than for Cadiz and Hendaye samples (multiple-comparison Tukey-type test, $p < 0.01$) (Table 6). Discriminant analysis of the cyst measurements revealed significant differences among all stations ($p < 0.001$) except between Cadiz and Hendaye ($p = 0.058$). A scatterplot of canonical scores (Fig. 3) highlighted a strong isolation of the sympatric association (Salses-Leucate) on Axis 1. The Fehmarn population was isolated on Axis 2. A Mantel test between genetic (Fig. 4a) and Mahalanobis (Fig. 4b) distances was significant ($r = 0.76$; $p = 0.04$).

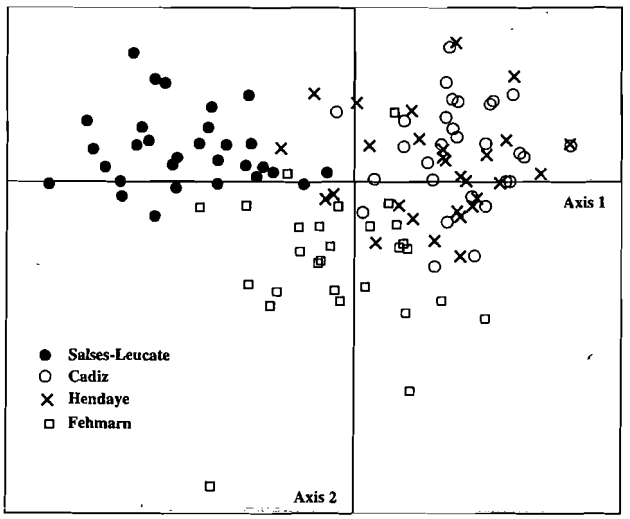

Fig. 3. *Pomatoschistus microps*. Discriminant analysis of individual histological measurements of metacercarial cysts for each sampling area

DISCUSSION

Our study compared the reaction of a naive host (*Pomatoschistus microps*) to a new strain of parasite with a local association of host-parasite system. This is close to Association 2 in our 'Introduction'. In our case, only the strain of parasite was new to the host species which (except for the Cadiz population) was naturally infested by this parasite species. Such a situation can be expected to occur often during the process of biological conservation, when species recolonize protected areas or when populations are displaced for aquaculture, or to increase genetic combinations.

Genetic comparison

The results of the genetic analysis indicate significant genetic variation among the 4 samples of *Pomatoschistus microps*. Such genetic differentiation was a prerequisite for our experiment, as geographic separa-

Table 5. *Pomatoschistus microps*. Estimation of deviation from Hardy-Weinberg expectation within samples (F_{IS}) and p-values (significance of the estimation) calculated by Markov chain method. **Bold**: indicates significant deficiency of heterozygotes (low heterozygosity). –: absent

Locus	Salses-Leucate		Cadiz		Hendaye		Fehmarn	
	F_{IS}	p	F_{IS}	p	F_{IS}	p	F_{IS}	p
ADA	0.005	**0.02**	0.02	0.23	0.01	**0.04**	0.025	0.54
GOT	−0.05	1	–	–	–	–	–	–
IDH	−0.01	1	0.12	0.08	0.37	**<0.01**	–	–
MPI	0.55	**0.03**	0.08	0.16	0.2	**0.02**	0.06	0.23
PEP B	–	–	0.27	0.09	–	–	–	–
PEP E	0.37	**0.01**	−0.01	1	–	–	–	–
PGD	0.05	0.54	–	–	–	–	–	–
PGI1	−0.14	0.90	0.05	0.40	−0.01	0.61	0.09	0.36
PGI2	–	–	0.05	0.46	0.18	0.24	0.65	**<0.01**
PGM1	0.30	**0.01**	0.27	**0.03**	−0.01	1	0.67	**<0.01**
PGM2	0.21	0.08	0.12	0.17	−0.02	1	–	–

Table 6. *Pomatoschistus microps*. Quantitative and qualitative results (means ± SE) of experimental infestation. N_i = mean initial number of encysted metacercariae; N_{+30} = mean number of encysted metacercariae after experimental infestation with 30 cercariae; S_{ind} = mean percentage of individual infestation success; I_{pop} = mean percentage of infestation success at population level (taking into account fish that died during experiment, *D*); *VM*: metacercarial estimated volume (10^{-3} mm^3); *CC*: cyst cavity estimated volume (10^{-3} mm^3); Interf: interface width (10^{-3} mm); *DC*: digestive caecum estimated volume (10^{-4} mm^3); *EB*: excretory bladder estimated volume (10^{-4} mm^3); SE: standard error

Area	N_i ± SE	N_{+30} ± SE	S_{ind} ± SE	I_{pop}	(*D*)	*VM* ± SE	*CC* ± SE	Interf ± SE	*DC* ± SE	*EB* ± SE
Salses-Leucate	13.6 ± 3.6	29.2 ± 5.0	39.9 ± 9.4	22.8	(12)	4.9 ± 0.3	6.6 ± 0.3	10.2 ± 0.4	2.1 ± 0.3	7.7 ± 0.8
Cadiz	0 ± 0	11.0 ± 1.3	36.7 ± 4.4	36.7	(0)	2.3 ± 0.1	3.5 ± 0.2	4.7 ± 0.3	2.0 ± 0.3	2.7 ± 0.3
Hendaye	3.9 ± 1.3	16.6 ± 1.9	41.0 ± 6.2	30.3	(7)	2.5 ± 0.2	3.7 ± 0.3	5.2 ± 0.3	1.1 ± 0.1	4.1 ± 0.6
Fehmarn	1.0 ± 0.5	9.4 ± 1.8	29.0 ± 5.7	14.7	(11)	3.8 ± 0.2	6.7 ± 0.4	6.2 ± 0.3	2.6 ± 0.6	6.5 ± 0.7

tion is not in itself sufficient to ensure local adaptation. Our results also revealed strong differentiation between the Mediterranean population and the other 3 populations. Two hypotheses could explain this: (1) It could be linked to the strong geographical isolation of the Mediterranean population, which is restricted mainly to enclosed coastal lagoons along the coast of France; this hypothesis could be confirmed by examining the genetic structure of populations in other lagoons in the southern part of France. (2) It might be a consequence of the paleobiogeographic history of the Mediterranean Sea during the last glaciation (Keigwin & Thunnel 1979); the Pleistocene drop in the sea level would have resulted in isolation of the Mediterranean populations of *P. microps*, as has been demonstrated for other fish species (Quignard 1978, Tirard et al. 1992).

Quantitative parasite success

We found no quantitative difference in the success of parasite infection between the 4 host populations. This result may reflect a lack of heritability of host suitability and/or of parasite infectivity (Grosholz & Ruiz 1995). Although it has been shown that this parasite species *Labratrema minimus* has a significant negative effect on its host's condition-factor (Faliex & Morand 1994), it seems likely that such effect puts little selective pressure on the host. Therefore, long-term association would not have induced 'co-evolutionary' adaptation in parasite success between sympatric and allopatric crosses in each region to a measurably different extent. Moreover, differences in generation times between host and parasite (the parasite generally having the shorter generation time: Price 1980, Lively 1989) has been invoked as an alternative explanation for local adaptation. However, *Pomatoschistus microps* has a short life span of 2 yr (Miller 1975) that should not be very different from the life span of the parasite (Maillard 1976), making it much more difficult to detect any evolutionary advantage the parasite might have over its local host. Finally, as this parasite species is opportunistic (i.e. it usually parasitizes different host species as second intermediate hosts [Maillard 1976]), it may infect most exposed hosts, especially when these belong to genetically close species. The result would be a lack of a quantitative difference in infective success, in terms of the number of penetrating parasites, between the various host populations.

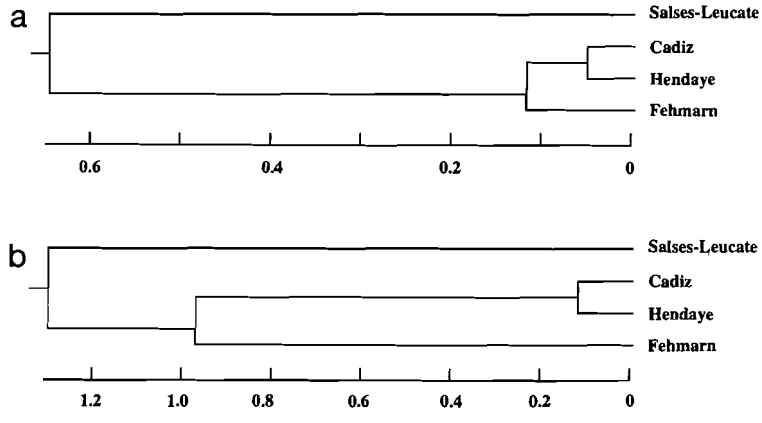

Fig. 4. *Pomatoschistus microps*. UPGMA dendrogram showing relationships between populations based on (a) genetic and (b) Mahalanobis distances, calculated using histological measurements of metacercarial cyst

Qualitative parasite success

However, as a result of the opportunistic behaviour of this parasite species, its qualitative success should vary among (1) different host species the parasite is able to infest, and (2) genetically different entities of the same host species. When the digenean cercariae penetrate the host, the fish hepatic cells are stored in the digestive caecum. During metacercarial development, the digestive caecum volume de-

creases and the wastes are collected in the excretive bladder of the metacercaria. The cyst wall is composed of 3 cell layers, of mixed origin (Faliex 1990), whose degree of infestation reflects the degree of interaction between host and parasite. Our results revealed a differential success of the parasite at the histological level: it is able to penetrate the host and to reach the liver, but it is unable to develop optimally in allopatric associations. This result seems to indicate a better relationship between sympatric parasite and host arising from a long-term association. A similar result was reported for a cestode of the striped bass (Sakanari & Moser 1990). Such differential development of metacercarial cysts in the liver could have selective consequences for both host and parasite. For the host, less-developed cysts would occupy less volume in the liver and might thus be less pathogenic; for the parasite, the survival of its second intermediate host would be indispensable to its own successful development prior to transfer to the final host.

Parasitism and conservation

Parasites are usually less well adapted and more virulent to a newly colonized host species (Toft & Karter 1990, Ewald 1995). Thus, the recolonisation of protected areas by new host species or the translocation of individuals (accidentally or intentionally) into new locations together with their parasites may induce new host-parasite associations and represent threats to the survival of native host populations. In turn, introduced populations would be faced with native parasite populations that could respond in novel ways in terms of infectivity and virulence, and abort their recolonisation success. When it occurs, local adaptation of host-parasite systems should be an important factor affecting biological conservation, as the performance and population growth of the parasite should increase over time as it adapts to its new local host. Our results as well as those of earlier studies on other host-parasite systems (Vera et al. 1990, Ballabeni & Ward 1993, Ebert 1994, Lively & Jokela 1996) seem to contradict the hypothesis of high virulence in new host-parasite associations, and indicate a need to reconsider the potential risk of introducing parasites with recolonizing host species. Nevertheless, the response of the host-parasite system will depend upon the structure of both host and parasite populations. For example, when host populations are isolated by distances or barriers sufficient to prevent parasite dispersal, gene flow may effect local differentiation between populations of both host and parasite (Mopper 1998). Moreover, fragmented host populations, arising either from translocation of individuals to new sites or from low densities of a rare or endangered species, will provide but a patchy environment for a parasite. This will reduce parasite gene flow and favor local adaptation (Peterson & Denno 1998). Such local adaptation of a parasite, when/if it occurs, should increase the likelihood of differential compatibility in allopatric pairings of host and parasite, and result in a longer term disequilibrium of the association. Populations of endangered species will be fragmented in isolated protected zones, and this should prove important in limiting the spread of any epidemic diseases that may occur (Dobson & May 1986, Packer et al. 1991).

Finally, lack of evidence of serious harm to their hosts by introduced parasite species does not automatically imply the absence of any deleterious effect (Mills et al. 1993), it merely underlines the limitations of our knowledge on the subject. It has long been thought that parasitic virulence results from misadaptation of the parasite to its host; this view of the host-parasite relationship has evolved during recent years (Bull 1994, Ebert 1994, Ewald 1995).

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