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Differential susceptibility to a trematode parasite among genotypes of the *Mytilus edulis/galloprovincialis* complex

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Summary

We show that parasitism by the trematode *Prosorhynchus squamatus* in parental and introgressed *Mytilus edulis/galloprovincialis* (Bivalvia) mussels occurs in individuals with a predominantly *M. edulis* genome. This result suggests that the restricted specificity of *P. squamatus* is dependent on genetic factor(s) present in *M. edulis*. Because of its strong pathogenic effects (i.e. total castration and possible death), this parasite may be a source of intense selection against *M. edulis* genomes when they are present in a site. As a consequence, it may favour the geographic extension of the *M. galloprovincialis* genome. Previous studies have indicated that, in hybrid zones, recombinant genotypes are more susceptible to parasitic infections than either parental genotype. We demonstrate that this is not the case for the *M. edulis/M. galloprovincialis* system, and that the parental genotype alone determines susceptibility.

1. Introduction

Recent studies of parasitism in hybrid zones (Sage *et al.* 1986; Whitham, 1989) indicated that introgressed hosts are unusually parasitized as compared to both parental species. Since no environmental or ecological peculiarities appear to make hybrids more liable to infection, it was suggested that introgressed genotypes are more susceptible to pathogens than parental genotypes, and that this higher-than-normal susceptibility to parasitism could, in reducing gene flow between the two hosts, contribute to maintaining their genetic identity.

In this article, we have studied the susceptibility of parental and introgressed *M. edulis/M. galloprovincialis* mussels to the Bucephalid trematode *Prosorhynchus squamatus* (Odhner, 1905). *Mytilus edulis* has a circum-polar distribution in the northern hemisphere and occurs as far south as North Africa (Fig. 1). *M. galloprovincialis* occupies warmer waters of Mediterranean, Adriatic and Black seas. However, morphological evidence indicates that it occurs sympatrically with *M. edulis* on the European Atlantic coasts, from the British Isles to Spain (Lewis & Seed, 1969; Seed, 1972) (Fig. 1). The two forms are known to hybridize and introgress in various proportions at sites in south-west England and Ireland (Gosling, 1984; Gosling & Wilkins, 1981; Skibinski *et al.* 1978; Skibinski & Beardmore, 1979).

P. squamatus has three successive hosts, lamellibranch molluscs, teleost fishes, and finally ichthyophagous teleost fishes as the definitive host (Chubrik, 1966). To date this parasite has been reported only in *M. edulis* (Chubrik, 1966; Matthews, 1972) in which it occurs as sporocysts and cercariae. While filtering water, the mussel is infested by a free stage of the parasite (Cheng, 1986).

The geographic distributions of *M. edulis* and *P. squamatus* overlap so that, in the zone in which *M. edulis* and *M. galloprovincialis* are sympatric, the parasite has potentially access to both these mussels as well as to their hybrids. At a microgeographic scale, the prevalence of *P. squamatus* in each mussel population is dependent on the occurrence of the definitive host in the site. Thus, the association between genotypes and parasitism can best be tested by submitting the different genotypes (*M. edulis*, *M. galloprovincialis* and introgressed mussels) to natural infestation in a single site and during the same period. The pattern of infectivity of *P. squamatus* in the different genotypes should indicate the limits of parasite specificity and host susceptibility, as well as

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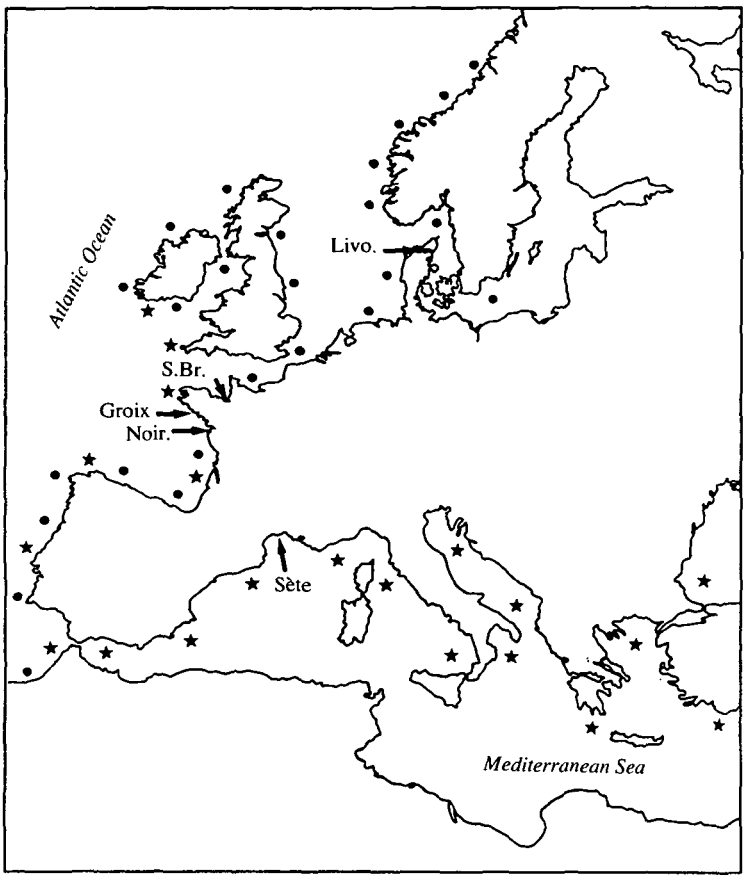

Fig. 1. Location of sampling sites (Livo, St Brieuc (S.Br.), Groix, Noirmoutier (Noir.) and Sète), and geographic distributions of *M. edulis* (●) and *M. galloprovincialis* (★) on European coasts according to Lubet *et al.* (1984).

the selective constraints exerted on the host populations by the parasite.

2. Material and methods

(i) *Mussel populations*

Samples were collected in five localities. Samples from Livo (Denmark) in the North Sea and from Sète (France) in the Mediterranean were used as references for *M. edulis* and *M. galloprovincialis*, respectively, since these two species are not sympatric in these areas. Mussels from the zone of sympatry were sampled in three populations off the French coasts according to their morphological characters (Seed, 1972). Mussels from Noirmoutier displayed *M. edulis* characters, and those from St Brieuc displayed *M. galloprovincialis* characters. In Groix, there are mussels with morphological characters of both taxa and in addition mussels with intermediate characters (Coustau *et al.* 1990).

(ii) *Experimental design*

Samples from the zone of sympatry were collected as brood (3–8 mm length) in July 1987, and were immediately implanted at the same depth in the mussel biotope of the island of Groix where *P. squamatus* is present (Coustau *et al.* 1990). Three control batches were also implanted at the same time in a locality (Trinité sur Mer, France) known to be free of parasites, in order to verify the healthy status of the experimental broods. After one year, the experimental and control mussels were dissected and examined for parasitism under a binocular microscope. No parasites were found in the controls, but the Trematode was present in the form of branched sporocysts in the mantle and visceral mass of some of the experimental mussels.

(iii) *Genotype characterization of mussels*

The genotypes of experimental mussels (either healthy or parasitized) were determined at five partially diagnostic loci (Skibinski, 1983; Beaumont *et al.* 1989), namely esterase D (Est D), aminopeptidase, glucose phosphate isomerase, octopine dehydrogenase and mannose phosphate isomerase (MPI), using horizontal starch gel electrophoresis (Pasteur *et al.* 1988). The experimental mussels examined included 86 individuals from Noirmoutier, 42 from St Brieuc and 90 from Groix, as well as 40 *M. edulis* from Livo and 56 *M. galloprovincialis* from Sète as controls for *M. edulis* and *M. galloprovincialis* genotypes, respectively.

(iv) *Data analysis*

Data were analysed by several methods including a factorial correspondence analysis (FCA) (Benzécri & Coll, 1973; Lebart *et al.* 1984) adapted for allozyme characters (She *et al.* 1987). Each individual is described for each allele by the values 2, 1, or 0 according to whether it possesses 2 (homozygote), 1 (heterozygote), or 0 copies of the considered allele. Individuals are then processed as active variables using FCA. The allelic frequencies of each sample are processed as supplementary variables, i.e. they are positioned in the figure but they are not used to compute the factor axes. They represent the centre of gravity of each sample and describe the differentiation between samples.

Statistical significance of the differentiation between two samples is tested by the *G* test (Scherrer, 1984; Sokal & Rolhf, 1969) applied to a contingency table (samples × alleles) where the samples are described by their absolute allelic frequencies.

3. Results

(i) *Genetic characterization of mussel samples*

Comparison of the allelic frequencies at five loci (Table 1) confirmed that allopatric *M. galloprovincialis* (Sète sample) and *M. edulis* (Livo sample) cannot be discriminated by single diagnostic alleles, in agreement with previous reports (Skibinski, 1983; Beaumont *et al.* 1989). However, the differences in frequency of some alleles are large enough to permit discrimination of the two species when two or several loci are considered simultaneously. Thus, when considering the MPI and Est D loci, mussels from Sète and Livo can be classified in two non-overlapping groups of genotypes characterized by the number of '*M. galloprovincialis* alleles' (Fig. 2). Similarly, the factorial correspondence analysis which considers genotypes at the five loci studied clearly shows that Sète and Livo individuals belong to two non-overlapping clusters (Fig. 3*a*).

Among the three experimental samples from the zone of sympatry, those from Noirmoutier and St Brieuc appear genetically very close to those from Livo and Sète, respectively (Table 1, Fig. 3*a*, *b*). A comparison of the genotype structures at MPI and Est D loci (Fig. 2) disclosed, however, a larger number of individuals with alleles typical of *M. edulis* in the St Brieuc sample than in the Sète sample. These *M. edulis* alleles are mostly associated with *M. galloprovincialis* alleles (Figs. 2, 3*b*), suggesting that *M. galloprovincialis*/*M. edulis* introgression has occurred in the past in St Brieuc. The sample from Groix displayed allelic frequencies intermediate between those of Sète and Livo (Table 1) and its individuals are distributed over the whole range of genotypic variation of both species in the factorial correspondence analysis (Fig. 3*a*). This situation is due to the presence of genotypes characteristic of both species as well as of genotypes formed by interbreeding between these species (Fig. 2). In contrast to St Brieuc, introgression appears to be still going on in Groix.

(ii) Prosorhynchus squamatus *parasitism*

Parasitism by *P. squamatus* was determined for each individual mussel that was examined electrophoretically. Results showed that parasitism did not occur at random among mussels (Fig. 3*c*), for none of the St Brieuc mussels (*M. galloprovincialis* type) were parasitized, whereas a significant number of individuals of the Groix and Noirmoutier samples were (Fig. 3*c*). No genetic difference between healthy and parasitized individuals was observed in the Noirmoutier (*edulis* type) sample (*G* test: $G^2 = 10{\cdot}2$, D.F. = 19; $P > 0{\cdot}05$). In contrast, in the sample of Groix, which included numerous introgressed individuals, the genotypes of healthy and parasitized mussels were significantly different (*G* test: $G^2 = 73$, D.F. = 20; $P \ll 0{\cdot}001$), the parasitized group being almost entirely of the *edulis*

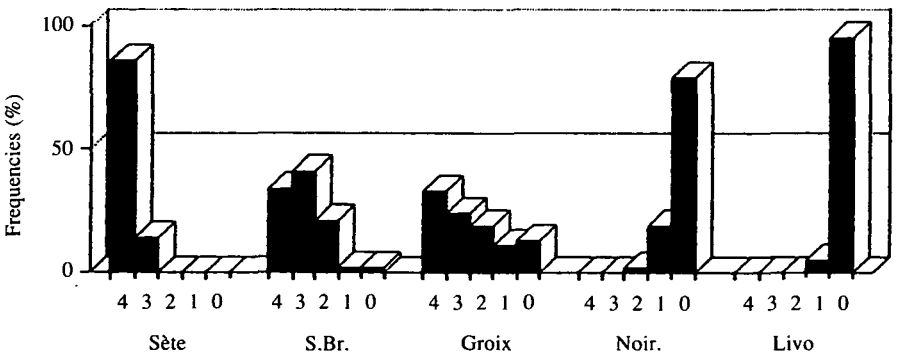

Fig. 2. Frequencies of mussels displaying 4, 3, 2, 1, or 0 '*M. galloprovincialis* alleles', in the samples from Sète (= typical *M. galloprovincialis*), from Livo (= typical *M. edulis*), St Brieuc (S.Br.), Groix, and Noirmoutier (Noir.). *M. galloprovincialis* 'synthetic alleles' consist of alleles 1 and 2 for Est D, and of allele 1 for MPI. On the abcissa are indicated the number of '*M. galloprovincialis* alleles', and on the ordinates the relative frequencies observed in each sample.

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Table 1. *Allelic frequencies at Est D, LAP, PGI, ODH, and MPI loci in* M. galloprovincialis *from Sète,* M. edulis *from Livo, and in the three experimental samples from St Brieuc (S.Br.), Groix and Noirmoutier (Noir.).* n *is the number of genes sampled. Alleles are numbered in order of increasing anodal mobility*

			Alleles						
Locus	Sample	*n*	1	2	3	4	5	6	7
Est D	Sète	112	0·03	0·93	0	0·04	0		
	S.Br.	84	0	0·81	0	0·19	0		
	Groix	180	0·03	0·62	0·02	0·31	0·02		
	Noir.	172	0	0·04	0·02	0·91	0·03		
	Livo	80	0·01	0	0·01	0·98	0		
LAP	Sète	112	0	0·02	0·05	0·31	0·01	0·56	0·05
	S.Br.	84	0	0·05	0·02	0·47	0·02	0·42	0·02
	Groix	180	0·03	0·08	0·02	0·48	0·01	0·37	0·01
	Noir.	172	0·02	0·09	0·01	0·66	0·05	0·16	0·01
	Livo	80	0·01	0·26	0	0·53	0·01	0·16	0·03
PGI	Sète	112	0	0	0·02	0·78	0·15	0·05	0
	S.Br.	84	0·01	0·01	0·05	0·54	0·25	0·13	0·01
	Groix	180	0	0·01	0·05	0·39	0·32	0·22	0·01
	Noir.	172	0	0·01	0·05	0·27	0·19	0·47	0·01
	Livo	80	0	0·02	0·05	0·23	0·18	0·50	0·02
ODH	Sète	112	0	0·09	0·22	0	0·69		
	S.Br.	84	0·01	0·47	0·26	0	0·26		
	Groix	180	0·01	0·38	0·41	0	0·21		
	Noir.	172	0·01	0·07	0·88	0	0·04		
	Livo	80	0	0·05	0·76	0·01	0·18		
MPI	Sète	112	0·96	0·03	0·01				
	S.Br.	84	0·69	0·31	0				
	Groix	180	0·61	0·38	0·01				
	Noir.	172	0·02	0·96	0·02				
	Livo	80	0·04	0·96	0				

type. These observations are confirmed on the projections of the factorial correspondence analysis by the position of the centres of gravity of healthy and parasitized mussels (Fig. 3*c*). In the Noirmoutier sample, these centres are almost superimposed, whereas in the Groix sample they are clearly distinct. To ascertain that the position of the centre of gravity of the 15 parasitized mussels from Groix within the *M. edulis* cluster (Fig. 3*c*) cannot be due to randomness, the centres of gravity of 100 trials with 15 mussels chosen at random (without replacement) within the Groix sample were positioned (Fig. 3*d*). None was identical to that of the parasitized sample, indicating that the genetic difference between parasitized and healthy mussels in the Groix sample is not an artefact ($P < 0{\cdot}01$).

4. Discussion

Our observations indicate that, in an environment in which mussels have an equal probability of being parasitized by a free-living *P. squamatus* stage (Cheng, 1986), those with an *M. edulis* genotype, either 'pure' or introgressed, are preferentially parasitized. Susceptibility to *P. squamatus* is, therefore, related to a still unidentified characteristic of the *edulis* genome.

The difference in susceptibility observed between *M. galloprovincialis* and *M. edulis*, two genetically very close taxa (Skibinski *et al.* 1980), may be ascribed either to mechanisms related to immunity, or to peculiar mesologic requirements of the parasite in its host. In either case, this differential susceptibility should provide a strong uni-directional selection in natural introgressed populations in favour of *M. galloprovincialis*.

The low viability of the *M. edulis* genotypes observed in introgressed populations off the coast of Britain has been attributed until now to unfavourable physical factor(s) (Skibinski, 1983; Gardner & Skibinski, 1988). The present work suggests that it could also be due to host–parasite interactions, since *P. squamatus* has strong pathogenic effects inducing castration and possible subsequent death (Coustau *et al.* 1990). Our study provides an example where susceptibility is clearly related to a single parental genome (*M. edulis*).

In the genomic confrontation that occurs when two taxa hybridize (Barton & Hewitt, 1985; Hewitt, 1988), the selective pressure exerted by a parasite may have widely different consequences. In some cases, parasitism may help to maintain a 'tension zone' (Key, 1981; Barton & Hewitt, 1989) by reducing the fitness

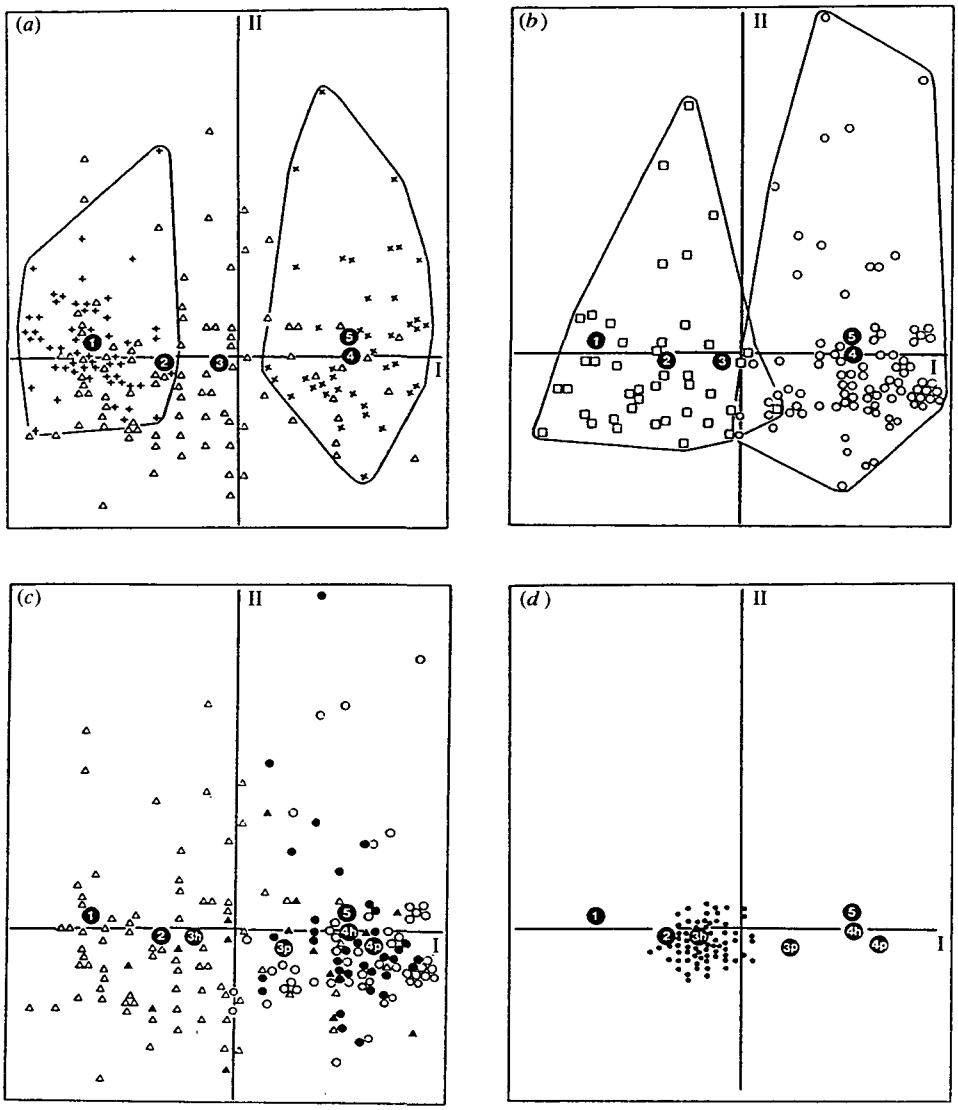

Fig. 3. Genetic variability and susceptibility to *Prosorhynchus squamatus* analysed by a factorial correspondence analysis in mussel samples from Sète (1), St Brieuc (2), Groix (3), Noirmoutier (4), and Livo (5). The first (I) and second (II) factor axes explain, respectively, 22 and 8% of the global variability of the five samples. The centres of gravity of each sample are identified by the population number. They characterize an almost unidimensional *M. galloprovincialis*/*M. edulis* gradient along the first factorial axis. (*a*) Projection of *M. galloprovincialis* from Sète (+), *M. edulis* from Livo (×), and mussels from Groix (△). (*b*) Projection of St Brieuc (□) and Noirmoutier (○) individuals. (*c*) Projection of Groix (△) and Noirmoutier (○) mussels considering healthy (△, ○) and *P. squamatus* parasitized (▲, ●) individuals. Centres of gravity are designated by 4h and 4p for Noirmoutier and by 3h and 3p for Groix, 'h' designating healthy and 'p' parasitized mussels. (*d*) Projection of the centres of gravity of 100 groups of 15 genotypes chosen at random among the 90 mussels from Groix (●). Among the 100 centres, 66 are projected, the other 34 being superimposed. The centres of gravity of the samples studied are represented as in (*c*).

of the hybrids, with respect to that of either parent, as observed in house mice parasitized by intestinal worms (nematodes and cestodes) (Sage *et al.* 1986) or in cottonwood parasitized by aphids (Whitham, 1989). In other cases, as described here, the selective pressure exerted by a parasite may favour the non-susceptible genome enabling it to displace the competing genome and, thus, to extend its distribution to its physiological limits.

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