REVIEW

Heterozygote deficiency in the mussel *Mytilus edulis* species complex revisited

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ABSTRACT: In order to understand the phenomenon of heterozygote deficiency (F_{is}) in marine molluscs, all the relevant literature concerning this phenomenon in the *Mytilus edulis* species complex was reviewed. Due to large heterogeneity in methods of data analysis, in particular for the choice of the estimator measuring heterozygote deficiency and for the choice of the testing procedure, no clear overview was possible. To overcome this problem, similar estimators and tests were used to analyze or re-analyze some additional large data sets from the Baltic (*M. trossulus*), France (*M. galloprovincialis*), England (*M. edulis*), Atlantic USA (*M. edulis*) and Pacific USA (*M. trossulus*). Large and significant heterozygote deficiencies exist in these data sets. Estimated F_{is} ($\hat{F}_{is}$) values are generally higher for *Lap* than for other loci, consistent with an effect of selection. No other significant variation of $\hat{F}_{is}$ values across loci within each data set or across data sets for each locus was detected, however no specific test has been designed for this null hypothesis. The possible contribution of a Wahlund effect to explain the heterozygote deficiency is discussed. It is likely that there is no unique explanation of heterozygote deficiency (such as a Wahlund effect or selection) in *M. edulis* or in other organisms, and that species-specific, locus-specific or population-specific explanations are to be sought.

KEY WORDS: *Mytilus* · Population genetics · Heterozygote deficiency · Allozyme

INTRODUCTION

Heterozygote deficiency in marine bivalves is described in a large body of literature, and several explanations have been proposed: first, a spatial or temporal Wahlund effect (Wahlund 1928) due to the incorporation of individuals from differentiated populations or cohorts in the same sample; second, a particular phenomenon at the chromosomal level, such as aneu-ploidy or parental imprinting; third, the presence of selection at the locus studied (or at a nearby locus in linkage disequilibrium with the marker); fourth, the frequent presence of null alleles (for reviews see e.g. Singh & Green 1984, Zouros & Foltz 1984, Foltz 1986, Zouros et al. 1988, Gaffney et al. 1990). Some explanations have been ruled out for a few particular species, but no consensus has emerged at present to explain heterozygote deficiencies in marine bivalves.

The phenomenon of heterozygote deficiency in marine bivalves was described for the first time in the mussel *Mytilus edulis*, and concerned only 1 locus (*Lap*) (Milkman & Beaty 1970), in a geographic location where strong selection occurs for this locus and generates heterozygote deficiency each generation (Koehn

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et al. 1980, Koehn & Immermann 1981, Koehn & Siebe-
naller 1981, Hilbish et al. 1982, Hilbish 1985, Hilbish
& Koehn 1985). Studies have since then been ex-
panded to other loci, other populations and other bi-
valve species, and, apparently, heterozygote defi-
ciency is a widespread phenomenon in this group of
marine organisms (Singh & Green 1984, Zouros & Foltz
1984).

Mytilus edulis has remained one of the few species
thoroughly studied for the problem of heterozygote
deficiency. The taxonomy and the various members of
this species complex have been studied (the *M. edulis*
species complex contains 3 members: *M. edulis*, *M.
trossulus* and *M. galloprovincialis*). The locations of
hybrid or contact zones or secondary intergradation
are relatively well known in some areas (e. g. Skibinski
et al. 1978, 1983, Skibinski & Beardmore 1979, Skibin-
ski 1983, Varvio et al. 1988, Coustau et al. 1991, Koehn
1991, McDonald et al. 1991, Väinölä & Hvilsom 1991,
Sarver & Foltz 1993, Sanjuan et al. 1994, Bates & Innes
1995, Quesada et al. 1995a, b, Rawson & Hilbish 1995).
Hence, reports of heterozygote deficiency from such
areas can be assigned to a Wahlund effect. These mus-
sels are also studied for reasons other than heterozy-
gote deficiency, so that independent data sets are
available to circumvent a possible publication bias.

From the large amount of information published on
this particular species, is it possible to draw general
conclusions on the significance of heterozygote defi-
ciency? The purpose of this paper is to review the
accessible data concerning heterozygote deficiency in
the *Mytilus edulis* complex. Due to large hetero-
geneities in methods of data analysis, in particular for
the choice of the estimator measuring heterozygote
deficiency and for the choice of the testing procedure,
no clear overview was possible. To overcome this prob-
lem, similar estimators and tests were used to analyze
or re-analyze some large data sets.

MATERIALS AND METHODS

Revisiting the *Mytilus edulis* literature. The 26
years of publication on heterozygote deficiency in the
Mytilus edulis complex were divided for convenience
into 2 periods, from the beginning (1970) to 1984, and
from 1985 to the latest papers (1996). Two review arti-
cles from 1984 (Singh & Green 1984, Zouros & Foltz
1984) ensure that no old papers were overlooked. A lit-
erature search on accessible databases was performed
for recent publications, but some papers may have
remained unnoticed. All papers which display a mea-
sure or a test of heterozygote deficiency in at least 1
sample from a natural population were retained for
further examination. However, papers devoted to the
study of hybrid zones were not considered, unless pure
populations were also studied (e.g. as in Skibinski et
al. 1983).

Data sets. Altogether 5 data sets were analyzed or
re-analyzed. The first was a Baltic data set from an
MSc thesis (Väinölä 1985). Eighteen samples from the
northern part of the basin (Finland and Estonia), all
located at least 750 km from the Öresund hybrid zone
(Väinölä & Hvilsom 1991), were selected for analysis
from a larger data set. Genotypes of a maximum of
3031 *Mytilus trossulus* mussels were available at
4 loci (*Lap, Ap, Pgi* and *Idh*). The second was a
French data set from a university report by F.T.
(Thomas 1993) which consisted of 6 samples of *M.
galloprovincialis* from the Mediterranean (Langue-
doc-Roussillon) and the Atlantic (Basque area). One of
the Atlantic samples (Vieux Boucaux) was removed
on the grounds that it was too close to a *M. edulis/gal-
loprovincialis* hybrid zone (Coustau et al. 1991, San-
juan et al. 1994) leaving only 5 samples in the data
set. Genotypes of a maximum of 143 *M. galloprovin-
cialis* mussels were available for 7 loci (*Lap, Pgi, Pep-
A, Pep-D, Mpi* and *Est-D*). The third was an English
data set published by Ahmad & Hedrick (1985) for
studies of linkage disequilibrium. Two samples of
1000 *M. edulis* each were analyzed for 4 loci. Detailed
genotypes at both *Lap* and *Ap* were available for fur-
ther analyses. The fourth was an Atlantic American
data set of *M. edulis* analyzed by Koehn & Gaffney
(1984) and kindly provided by P. M. Gaffney upon
request. This data set consists of 1 sample of 8 size
classes of the same age analyzed at 5 loci (*Lap, Ap,
Pgm, Odh, Pgi*). Although heterozygote deficiency
was measured (but not tested) in their paper, the pur-
pose of Koehn & Gaffney's work was to analyze the
growth/heterozygosity relationship of *M. edulis*, not
heterozygote deficiency. The fifth was a Pacific Amer-
ican data set published by Johnson & Utter (1973) for
the study of the genetics of 1 electrophoretic locus
(aspartate amino transferase, or *Aat*). Six samples rep-
resenting 644 individuals were analyzed at 1 locus
(*Aat*). The correct scientific name is certainly *M.
trossulus*, and not *M. edulis* as stated by the authors
(see e.g. McDonald et al. 1991).

Statistics. Hardy-Weinberg equilibrium (HWE) was
tested using the exact U-score test of Rousset & Ray-
mond (1995), when the alternative hypothesis (H_1) is
heterozygote deficiency. This test has several advan-
tages. First, it is an exact test, so that low sample size or
low allelic frequency is not a problem (in contrast to
tests based on asymptotic distributions). Second, the
power of this test (when H_1 is true) is close to the max-
imum possible. Third, this test can be readily extended
to multiple samples, the null hypothesis becoming
'random association of gametes in each sample' (Rous-

set & Raymond 1995). A complete enumeration algorithm (Louis & Dempster 1987) was used whenever possible to compute exact p-values, or a Markov chain method (Guo & Thompson 1992) was used for their unbiased estimates. In such cases, chains were at least 20000 steps long (up to 100000 when greater precision was needed), with 1000 steps of dememorization (see Guo & Thompson 1992 for details). Heterozygote deficiency F_{is} was measured using either the F_{is} estimator f_c of Weir & Cockerham (1984) or f_T of Robertson & Hill (1984). The latter has a minimum variance when $F_{is} = 0$ and is also monotonously related to the statistics used to define the rejection zone of the exact U-score test (see Rousset & Raymond 1995). The former could easily be extended to have global F_{is} estimates. For all these computations (tests and measures), the Genepop (version 2.0) software was used (Raymond & Rousset 1995).

RESULTS

Revisiting the *Mytilus-edulis*-complex literature

A total of 20 papers were analyzed. The occurrence of significant heterozygote deficiency in the most-studied loci (Table 1) is considered first. All studies except 1 (which is devoted to the genetics of the locus *Aat*) include the locus *Lap*. Due to the strong selection acting at this locus in some geographic areas, *Lap* has been analyzed separately. The 3 most widely analyzed loci are *Pgm*, *Odh* and *Pgi*. Significant and non-significant departures from Hardy-Weinberg equilibrium are reported for these 3 loci in Table 1, although it is difficult to extract an overall picture due to the variety of situations and approaches used. A more detailed analysis on this body of literature is needed.

Table 1. Literature from (A) 1970 to 1984 and (B) 1985 to 1996 consulted on heterozygote deficiency in the *Mytilus edulis* complex. Country where mussels were sampled, the number of samples analyzed and the number of loci examined are indicated. For the 3 most studied loci, *Pgm*, *Odh* and *Pgi* (*Lap* is excluded, see text), the significance level (Hardy-Weinberg; ns: p > 0.05; *p < 0.05; **p < 0.01; ***p < 0.001) of the test of heterozygote deficiency, as reported by the authors, is indicated. (?) indicates the result of the test was not clear; (–) indicates the locus was not examined

Country	Samples	No. of loci	Pgm	Odh	Pgi	Source
(A) 1970–1984						
USA	24	1	–	–	–	Milkman & Beaty (1970)
USA	4	2	–	–	–	Koehn & Mitton (1972)
USA	12	2	–	–	–	Mitton et al. (1973)
USA	6	1	–	–	–	Johnson & Utter (1973)
USA	16	1	–	–	–	Boyer (1974)
USA	150	6	–	–	ns	Koehn et al. (1976)
USA	18	1	–	–	–	Lassen & Turano (1978)
UK	68	11	?[a]	–	?[a]	Skibinski et al. (1983)
USA	8	5	?[b]	?[b]	?[b]	Koehn & Gaffney (1984)
(B) 1985–1996						
USA	3	5	?[c]	?[c]	?[c]	Diehl et al. (1985)
Ireland	3	4	–	–	ns	Gosling & Wilkins (1985)
Norway	13	30	ns	ns	ns	Fevolden & Garner (1986)
USA	1	7	**	ns	**	Zouros et al. (1988)
UK	1	8	[a]	[a]	[a]	Gentili & Beaumont (1988)
Ireland	1	5	*[d]	[e]	–	Gosling (1989)
Spain	9	4	–	*[f]	–	Sanjuan et al. (1990)
USA	6	5	?[b]	?[b]	?[b]	Gaffney (1990)
Iberian Peninsula	37	5	–	*[g]	–	Sanjuan et al. (1994)
UK	1	6	**	ns	ns	Fairbrother & Beaumont (1993)
Iberian Peninsula			?[h]	?[h]	*[i]	Quesada et al. (1995b)

[a]Test provided only for each allele
[b]Hardy-Weinberg not tested
[c]Test not indicated; p-values not given
[d]For both size classes
[e]p < 0.001 for small size classes, and not significant for large size classes
[f]Significant deficiency in 3 out of 5 samples
[g]Significant deficiency in half of the samples
[h]Details not given for samples outside the intergradation cline
[i]For samples outside the intergradation zone, 2 tests significant after Bonferroni correction

How was heterozygote deficiency measured?

Except for a few studies where heterozygote deficiency was not measured, 3 different measures are used: D, f_c and f_T (Table 2). D (or $-D$) is defined as $1 - H_o/H_e$, where H_o is the observed frequency of heterozygotes, and H_e is $1 - \sum p_j^2$ (p_j being the observed frequency of allele j in the sample). H_e is often designated (incorrectly, see Levene 1949) as the expected frequency of heterozygotes under HWE. f_c is the F_{is} estimator defined by Weir & Cockerham (1984), and f_T is the F_{is} estimator defined by Robertson & Hill (1984). The first measure is used in the majority of the studies, while the other 2 seem to be used only in recent publications (Table 2).

How was heterozygote deficiency tested?

Except for 6 studies in which no test of Hardy-Weinberg equilibrium is apparent, several types of test are used in the literature analyzed (Table 3). For asymptotic X^2 goodness of fit tests, which compare observed and 'expected' genotype counts, 2 different tests can be performed, depending on whether all genotypic classes (X^2-g test) or only 2 classes (homozygotes vs heterozygotes, X^2-h test) are considered. Another test, the asymptotic test proposed by Brown (1970), uses $\hat{F}$,

the maximum likelihood estimator of F_{is} for 2-allele cases (X^2-2 test). This test is a particular case of the test proposed by Li & Horvitz (1953) and Equation 1 of Brown (1970), where the variance of $\hat{F}$ is $\frac{1}{n}$ (n being the sample size), is always used. A third test is the asymptotic test described by Robertson & Hill (1984) (f_T-test). In addition miscellaneous tests which are not clearly described in the original publications are also used (see Table 3). The X^2-g and X^2-2 tests are widely used, and the others are used in only 1 (X^2-h) or few studies.

Studying heterozygote deficiency in 'independent' data sets

Baltic data set

The genotype of about 3000 mussels for the Lap, Ap and Pgi loci and of more than 1000 mussels for the Idh locus are analyzed. The mean sample size is 159 mussels, varying from a minimum of 64 to a maximum of 306. Measures of heterozygote departure from Hardy-Weinberg equilibrium are shown in Table 4. Multi-population estimated heterozygote deficiencies are positive for all loci, indicating some heterozygote deficiency, which is apparently higher for Lap. The overall estimate across all populations and all loci is positive and low when Lap is excluded.

Table 2. Measures of heterozygote deficiency in the *Mytilus edulis* complex used in the literature. Whether global estimates of F_{is} across loci or populations have been used is indicated. See 'Results' for further explanation

	$1-H_o/H_e$	f_c	f_T	None	Not indicated	Global estimates	Source
(A) 1970–1984							
					x	No	Milkman & Beaty (1970)
				x		No	Koehn & Mitton (1972)
				x		No	Johnson & Utter (1973)
				x		No	Mitton et al. (1973)
	x					No	Boyer (1974)
	x					Yes	Koehn et al. (1976)
	x					No	Lassen & Turano (1978)
	x					Yes	Skibinski et al. (1983)
	x					Yes	Koehn & Gaffney (1984)
(B) 1985–1996							
	x					Yes[a]	Gosling & Wilkins (1985)
	x					No	Diehl et al. (1985)
	x					No	Zouros et al. (1988)
					x[b]	Yes	Gentili & Beaumont (1988)
				x		No	Fevolden & Garner (1986)
	x					No	Gosling (1989)
		x	x			Yes	Sanjuan et al. (1990)
	x					No	Gaffney (1990)
	x					No	Fairbrother & Beaumont (1993)
		x	x			Yes	Sanjuan et al. (1994)
			x			No	Quesada et al. (1995b)

[a]Mean observed and expected heterozygosity
[b]Probably $1-H_o/H_e$

Table 3. Tests used in the literature concerned with heterozygote deficiency in the *Mytilus edulis* complex. Multiple indicates whether the significance level is adjusted to take into account multiple testing. Global indicates a global test is performed. See 'Results' for further explanations

	X^2-g	X^2-h	X^2-2	Other	None	Multiple	Global	Source
(A) 1970–1984								
					x	No	No	Milkman & Beaty (1970)
	x[a]					No	No	Koehn & Mitton (1972)
	x[a]					Yes[b]	Yes	Johnson & Utter (1973)
	x					No	No	Mitton et al. (1973)
	x				x	No	No	Boyer (1974)
				x[c]		No	Yes	Koehn et al. (1976)
			x			No	No	Lassen & Turano (1978)
			x			No	No	Skibinski et al. (1983)
					x	No	No	Koehn & Gaffney (1984)
(B) 1985–1996								
	x		x			No	No	Gosling & Wilkins (1985)
				x[d]		No	No	Diehl et al. (1985)
				x[d]		No	No	Zouros et al. (1988)
			x			No	No	Gentili & Beaumont (1988)
		x				No	No	Gosling (1989)
	x					No	No	Fevolden & Garner (1986)
				x[e]		No	No	Sanjuan et al. (1990)
					x	No	No	Gaffney (1990)
			x			Yes	No	Fairbrother & Beaumont (1993)
				x[e]		No	No	Sanjuan et al. (1994)
				x[e]		Yes	No	Quesada et al. (1995b)

[a]Alleles pooled
[b]Not explicitly indicated: 6 tests are performed, and a p > 0.01 is considered non significant
[c]Not enough information on the test used
[d]$D = 1 - H_o/H_e$ tested, no test indicated
[e]f_T-test, see text for details

Table 4. Measures of heterozygote deficiency for the Baltic data set (18 populations). Weir & Cockerham (1984) and Robertson & Hill (1984) estimators of F_{is} are given for each locus and each sample (respectively f_c and f_T). Estimates for all loci in each sample, for all samples for each locus, and for all samples and all loci are given (All). The locus *Lap* was excluded from 1 multi-locus measure (All – *Lap*)

Sample	*Lap* f_c	*Lap* f_T	*Ap* f_c	*Ap* f_T	*Pgi* f_c	*Pgi* f_T	*Idh* f_c	*Idh* f_T	All f_c	All – *Lap* f_c
1	+0.172	+0.218	−0.069	−0.059	−0.044	−0.018	−0.167	−0.168	+0.056	−0.082
2	+0.207	+0.172	+0.139	+0.167	−0.033	−0.021	+0.024	+0.024	+0.133	+0.057
3	+0.082	+0.040	+0.036	+0.057	−0.022	−0.009	−0.067	−0.067	+0.042	−0.015
4	+0.169	+0.142	−0.029	−0.014	+0.053	+0.061	+0.007	+0.007	+0.085	+0.003
5	+0.188	+0.208	+0.021	+0.003	+0.055	+0.033	−0.008	−0.008	+0.092	+0.015
6	+0.170	+0.185	+0.030	+0.039	−0.033	−0.012	−	−	+0.113	+0.012
7	+0.160	+0.157	+0.090	+0.088	−0.054	−0.023	−	−	+0.109	+0.031
8	+0.125	+0.118	+0.059	+0.062	+0.011	+0.072	−	−	+0.081	+0.039
9	+0.110	+0.082	−0.027	−0.058	−0.040	−0.017	−	−	+0.061	−0.032
10	+0.177	+0.182	−0.025	−0.063	−0.038	−0.013	−0.091	−0.092	+0.063	−0.050
11	+0.168	+0.143	−0.031	−0.054	+0.188	+0.240	+0.063	+0.063	+0.102	+0.055
12	+0.186	+0.160	+0.116	+0.163	+0.025	+0.066	−	−	+0.136	+0.077
13	+0.096	+0.111	+0.013	−0.011	+0.061	+0.018	−	−	+0.070	+0.031
14	+0.175	+0.183	+0.032	+0.027	−0.080	−0.038	+0.077	+0.077	+0.082	+0.020
15	−0.015	+0.024	−0.148	−0.102	−0.044	−0.019	−	−	−0.058	−0.119
16	+0.154	+0.162	+0.009	+0.040	+0.065	+0.044	−0.018	−0.019	+0.091	+0.016
17	+0.061	+0.038	−0.062	−0.013	−0.067	−0.030	−	−	+0.002	−0.064
18	+0.076	+0.079	+0.014	+0.020	−0.025	−0.026	+0.030	+0.030	+0.039	+0.012
All	+0.138		+0.010		+0.005		+0.007		+0.065	+0.008

Across populations, F_{is} estimates were significantly non-randomly ranked for each locus (Kendall test of concordance, $p < 10^{-5}$, first line of Table 5), suggesting that loci are subject to similar phenomena within each population. However, this trend was due to the high F_{is} value for *Lap*, as no significant ($p > 0.3$) effect was apparent when *Lap* was removed (Table 5). The median $\hat{F}_{is}$ value was not different for each locus (Kruskal-Wallis ANOVA by rank, $X^2 = 1.78$, df = 3, p = 0.62), except when *Lap* was also considered ($X^2 = 20.3$, df = 3, p = 0.0001).

Tests indicate an overall significant heterozygote deficiency, even when the *Lap* locus is removed from the data set (Table 6). Of the 4 loci, 3 were individually significant, although *Ap* displayed a marginally significant level. Hardy-Weinberg equilibrium cannot be rejected for *Idh* in this data set.

French data set

The genotypes of about 143 mussels for 7 loci (*Lap*, *Idh*, *Pgi*, *PepA*, *Pep-D*, *Mpi* and *Est-D*) are analyzed. The mean sample size is 24 mussels, varying from a minimum of 19 to a maximum of 35. Measures of heterozygote departure from Hardy-Weinberg equilibrium are shown in Table 7. Multi-population $\hat{F}_{is}$ are positive for all loci except *Pep-A* and *Mpi*, indicating some heterozygote deficiency, which is apparently higher for *Est-D* and *Idh*. The overall estimate across all populations and all loci is positive. Across populations, $\hat{F}_{is}$ was ranked randomly for each locus (Kendall test of concordance, $p > 0.20$, second line of Table 5), and the median $\hat{F}_{is}$ value was not different for each locus (Kruskal-Wallis ANOVA by rank, $X^2 = 7.0$, df = 6, p = 0.321).

Table 5. Measure and test of random ranking of $\hat{F}_{is}$ across populations for the various loci studied in each data set. The Kendall coefficient of concordance (W) and the corresponding p-value, computed according to Siegel & Castellan (1988), are indicated. Results are presented for all loci including and excluding *Lap*. A global test (Fisher's method) across data sets (All) is computed according to Fisher (1970). Bold characters indicate significant (p < 0.05) p-values

Data set	*Lap* included		*Lap* excluded	
	W	p	W	p
Baltic	0.676	**<10⁻⁵**	0.049	0.346
French	0.259	0.262	0.272	0.245
English	1.000	0.083	–	–
Atlantic USA	0.297	**<0.05**	0.262	0.098
Pacific USA	–	–	–	–
All	–	**<10⁻⁴**	–	0.143

Table 6. Testing heterozygote deficiency for the Baltic data set. Exact p-values are indicated for each locus in each sample. Unbiased estimates of exact p-values of the *U*-score test (Markov chain of 20000 steps for *Lap*, *Pgi* and *Idh*, and of 100000 steps for *Ap*) are given for global tests (All). All – *Lap* indicates that the *Lap* locus has been excluded for this multi-locus test. Bold characters indicate significant (p < 0.05) probabilities

Pop	*Lap*	*Ap*	*Pgi*	*Idh*	All	All – *Lap*
1	**10⁻⁴**	0.930	1	1	0.131	0.989
2	**0.001**	**0.004**	1	0.302	**0.003**	**0.044**
3	0.229	0.183	1	0.855	0.237	0.271
4	**0.011**	0.679	0.131	0.584	**0.029**	0.220
5	**0.001**	0.443	0.267	0.636	**0.016**	0.240
6	**0.001**	0.262	1	–	**0.019**	0.336
7	**0.006**	0.091	1	–	**0.032**	0.202
8	**0.013**	0.088	0.058	–	**0.004**	**0.009**
9	0.084	0.717	1	–	0.420	0.730
10	**0.003**	0.790	1	0.918	0.255	0.937
11	**0.004**	0.851	**0.001**	0.258	**<10⁻⁴**	**0.001**
12	**0.003**	**0.008**	0.093	–	**0.001**	**0.008**
13	**0.016**	0.517	0.201	–	0.075	0.293
14	**0.002**	0.315	1	0.268	0.056	0.405
15	0.358	1	1	–	0.760	0.974
16	**0.001**	0.223	0.111	0.699	**0.005**	0.120
17	0.222	0.581	1	–	0.521	0.783
18	0.075	0.331	0.739	0.431	0.230	0.453
All:	**<10⁻⁴**	0.050ᵃ	**0.016**	0.451	**<10⁻⁴**	**0.003**

ᵃA chain of 100000 steps was run for a better precision in this particular case, resulting in a standard error of the p-value of 0.002

Significant ($p < 0.05$) heterozygote deficiency is found for 3 loci (*Est-D*, *Idh* and *Pgi*) out of 7 (Table 8), and over all loci this deficiency is highly significant (p = 0.0029).

English data set

Ahmad & Hedrick (1985) presented genotypic data for 1000 mussels at 2 loci (*Lap* and *Ap*), in 2 populations. For the *Lap* locus, the measure of heterozygote deficiency is close to +0.1, and tests within each population or for both are highly significant (Table 9). For the *Ap* locus, the measure of heterozygote deficiency is lower and its sign is not congruent for both estimators, although the overall estimate is positive. Across populations, $\hat{F}_{is}$ was ranked randomly for each locus (Kendall test of concordance, $p > 0.05$, third line of Table 5), and the median $\hat{F}_{is}$ value was different between both loci (Kruskal-Wallis ANOVA by rank, $X^2 = 6.0$, df = 1, p = 0.0143). A significant deficiency ($p < 0.05$) is detected for the first population but not for the second ($p > 0.5$), and the overall test is not significant, although it is close to the significance level (Table 9).

Table 7. Measures of heterozygote deficiency for the French data set (2 Mediterranean and 3 Atlantic populations). Weir & Cockerham (1984) and Robertson & Hill (1984) estimators of F_{is} are given for each locus and each sample (respectively f_c and f_T). Estimates for all loci in each sample, for all samples for each locus, and for all samples and all loci are given (All)

Sample	Pep-A f_c	f_T	Est-D f_c	f_T	Lap f_c	f_T	Idh f_c	f_T	Mpi f_c	f_T	Pep-D f_c	f_T	Pgi f_c	f_T	All f_c
Mediterranean															
1	−0.014	+0.010	−0.030	−0.016	+0.160	+0.142	+0.366	+0.374	−0.065	−0.065	−0.050	−0.003	−0.045	+0.110	+0.027
2	−0.280	−0.188	+0.654	+0.684	+0.074	+0.047	−0.059	−0.060	−0.027	−0.028	+0.308	+0.129	−0.041	−0.042	+0.030
Atlantic															
3	+0.022	−0.004	+0.079	−0.027	+0.124	+0.045	+0.251	+0.256	−0.100	−0.101	−	−	+0.131	+0.028	+0.094
4	+0.123	+0.092	+0.341	+0.370	−0.055	−0.017	+0.081	+0.082	−0.070	−0.023	−0.052	+0.100	−0.037	+0.008	+0.033
5	−0.169	−0.124	−0.098	−0.050	+0.048	+0.009	+0.384	+0.398	+0.143	−0.003	−0.143	−0.064	+0.315	+0.188	+0.079
All	−0.025		+0.183		+0.076		+0.228		−0.033		+0.003		+0.065		+0.050

Table 8. Testing heterozygote deficiency for the French data set. Exact p-values are indicated for each locus in each sample. Unbiased estimates of exact p-values of the *U*-score test (Markov chain of 50 000 steps) are given for global tests (All). Bold characters indicate significant (p < 0.05) probabilities

Sample	Pep-A	Est-D	Lap	Idh	Mpi	Pep-D	Pgi	All
Mediterranean								
1	0.428	1	0.132	0.142	1	0.468	0.109	0.065
2	0.908	**<2 × 10⁻⁵**	0.479	1	1	0.097	0.777	0.211
Atlantic								
3	0.469	0.298	0.271	0.190	1	−	0.332	0.232
4	0.115	**0.026**	0.661	0.526	1	0.106	0.422	**0.017**
5	0.896	1	0.433	0.125	0.259	1	**0.021**	0.150
All	0.628	**0.027**	0.148	**0.011**	0.631	0.180	**0.022**	**0.003**

Atlantic American data set

The unique 1 cohort sample analyzed by Koehn & Gaffney (1984) is divided into 8 size classes. Measures of heterozygote deficiency are shown Table 10 and indicate a relatively high deficiency (overall estimate: +0.128), confirming their previous claim. The negative correlation between the measure of heterozygote deficiency and size class is confirmed using Weir & Cockerham's estimator (Kendall correlation = −0.762, n = 8, p < 0.05). $\hat{F}_{is}$ was not randomly ranked across size classes for each loci (Kendall test of concordance, p < 0.05, Table 5), although this effect disappeared when *Lap* was removed (Kendall test of concordance, p > 0.1, fourth line of Table 5). The median $\hat{F}_{is}$ value was not different for each locus (Kruskal-Wallis ANOVA by rank, X^2 = 3.0, df = 4, p = 0.558), except when *Lap* was also considered (X^2 = 11.0, df = 4, p = 0.0266). Hardy-Weinberg equilibrium was rejected (due to significant heterozygote deficiency) for all loci except *Ap* (Table 11).

Pacific American data set

Out of 6 samples, 4 display a high (f_t or f_c > 0.09) and significant (p < 0.02) deficiency of heterozygotes (Table 12). A slight heterozygote excess is displayed by 1 sample, and for another there is either an excess or a deficiency depending of the estimator used. No significant (p > 0.06) heterozygote deficiency is found in these 2 samples. Overall, there is a high (f_c = 0.162) and significant (p < 5 × 10⁻⁵) deficiency of heterozygotes (Table 12).

Some loci are studied in more than 1 data set, so that a comparative analysis is possible. For each locus, the median of the distribution of $\hat{F}_{is}$ is not different across data sets (Kruskal-Wallis 1-way ANOVA, *Lap*: X^2 = 6.19, df = 3, p = 0.103, Baltic, French, English, Atlantic USA data sets; *Ap*: X^2 = 3.42, df = 3, p = 0.331, Baltic, French, English, Atlantic USA data sets; *Pgi*: X^2 = 3.06,

Table 9. Measures and tests of heterozygote deficiency for the English data set (2 populations). Weir & Cockerham (1984) and Robertson & Hill (1984) estimators of F_{is} are given for each locus and each sample (respectively f_c and f_T). Estimates for all samples for each locus are given ('All'). Unbiased estimates of exact p-values of the *U*-score test (Markov chain of 200 000 steps for each locus in each sample and of 100 000 steps for multi-sample tests) are also given (test). Bold characters indicate significant (p < 0.05) results

Sample	Lap f_c	f_T	Test	Ap f_c	f_T	Test
Pendine	+0.076	+0.077	**<5 × 10⁻⁶**	+0.017	+0.036	**0.027**
Aberavon	+0.109	+0.051	**0.001**	+0.037	−0.002	0.518
All	+0.092		**<10⁻⁵**	+0.027		0.060

Table 10. Measures of heterozygote deficiency for the Atlantic American data set (1 cohort, 8 size classes). Weir & Cockerham (1984) and Robertson & Hill (1984) estimators of F_{is} are given for each locus and each sample (respectively f_c and f_T). Estimates for all loci in each samples, for all samples for in each locus, and for all samples and all loci are given (All)

Size class (mm)	Lap f_c	f_T	Ap f_c	f_T	Pgm f_c	f_T	Odh f_c	f_T	Pgi f_c	f_T	All f_c
4–6	+0.327	+0.302	+0.103	+0.087	+0.466	+0.564	−0.040	−0.024	+0.157	+0.107	+0.235
6–8	+0.160	+0.124	+0.074	+0.055	+0.517	+0.383	−0.054	−0.030	+0.073	−0.011	+0.148
8–10	+0.199	+0.202	+0.077	+0.068	+0.298	+0.306	+0.358	+0.136	+0.120	+0.079	+0.180
10–12	+0.227	+0.208	−0.071	−0.104	−0.002	+0.002	+0.225	+0.098	+0.334	+0.244	+0.157
12–14	+0.042	−0.011	−0.014	−0.018	−0.013	−0.030	+0.194	+0.040	+0.010	+0.011	+0.025
14–16	+0.130	+0.044	−0.036	−0.010	+0.193	+0.160	+0.069	+0.043	+0.050	+0.038	+0.065
16–18	+0.122	+0.110	−0.006	−0.015	+0.175	+0.093	+0.132	+0.089	+0.113	+0.028	+0.096
18–20	−0.032	−0.051	+0.094	+0.091	+0.030	+0.056	+0.444	+0.265	−0.065	−0.079	+0.061
All	+0.158		+0.026		+0.236		+0.195		+0.114		+0.128

Table 11 Testing heterozygote deficiency for the Atlantic American data set. Unbiased estimates of exact p-values of the U-score test (Markov chain of 100000 steps for each locus in each sample and of 50000 steps for multi-sample tests) are given. Bold characters indicate significant (p < 0.05) probabilities

Size class (mm)	Lap	Ap	Pgm	Odh	Pgi	All
4–6	**0.001**	0.135	**<10⁻⁵**	1	**0.045**	**<2 × 10⁻⁵**
6–8	0.057	0.238	**10⁻⁴**	1	0.554	**0.006**
8–10	**0.005**	0.188	**0.002**	0.056	0.111	**<2 × 10⁻⁵**
10–12	**0.006**	0.913	0.610	0.076	**0.001**	**0.003**
12–14	0.533	0.570	0.639	0.190	0.429	0.475
14–16	0.296	0.555	**0.030**	0.378	0.280	0.053
16–18	0.071	0.598	0.114	0.137	0.319	**0.036**
18–20	0.702	0.164	0.305	**0.035**	0.806	0.197
All	**<2 × 10⁻⁵**	0.261	**<2 × 10⁻⁵**	**0.019**	**0.004**	**<2 × 10⁻⁵**

$df = 2$, $p = 0.216$, Baltic, French, Atlantic USA data sets; Idh: $X^2 = 0.379$, $df = 1$, $p = 0.538$, Baltic, French data sets).

DISCUSSION

The subject of heterozygote deficiency in marine bivalves has attracted a large number of investigations, although no consensus exists among workers in this area. The number of species considered, the different statistical tools used, and the variety of geographical areas sampled and loci analyzed do not facilitate the comparison of the numerous papers on the subject. Even when only 1 species is considered (here the species complex Mytilus edulis), it is difficult to appreciate whether or not there is a general trend for heterozygote deficiency due to large variety in methods of data analysis (e.g. Table 1). Re-analysing pub-

lished data is rarely possible because genotypic data are seldom available. This aspect is problematic in population genetics, as better estimators and more adequate statistical tests are continuously appearing in the literature. For example, adequate estimators (at least understandable definitions) of departure from Hardy-Weinberg proportions, such as f_c or f_T (Robertson & Hill 1984, Weir & Cockerham 1984), were not available in the literature before 1984. Also, exact tests are now more common due to the recent development of computer power and of the occurrence of fast algorithms. But these exact tests require, in general, count data of genes or genotypes, which can rarely be reconstructed from published frequency data.

How do previous F_{is} estimators and tests affect our appreciation of heterozygote deficiency?

The most commonly used F_{is} estimator is the quantity $1 - H_o/H_e$ (Table 2), which is the maximum likelihood estimate (MLE) for the 2-allele case (Brown 1970). For more alleles, this quantity is not the MLE and it underestimates heterozygote deficiency (Curie-Cohen 1982). Other F_{is} estimators, such as f_c (Weir & Cockerham 1984) or f_T (Robertson & Hill 1984), are used only in 3 recent studies. Apparently most authors were not convinced of the utility of 'a standard set of formulae, clearly derived as estimators for well defined parameters' (Weir & Cockerham 1984).

All the tests used are concerned with the same null hypothesis, i.e. Hardy-Weinberg equilibrium. However, they do not have the same power with respect to a specific alternative hypothesis (H_1): their probabilities of rejecting the null hypothesis differ when H_1 is true. There are various ways to depart from Hardy-Weinberg equilibrium, and heterozygote excess or deficiency is only one possibility. A departure from

HWE could be the result of deficiency or excess of some particular genotypes (for example due to selection) without necessarily affecting a global heterozygote deficiency. The exception is the 2-allele case, where any departure from HWE could be explained as a heterozygote excess or deficiency. This is because, with only 2 alleles, gene frequencies and 1 F_{is} parameter completely describe the genotypic composition of a population. There have been repetitive warnings in the literature on the frequent confusion between testing HWE and testing heterozygote deficiency compared to HWE (see e.g. Pamilo & Varvio-Aho 1984, Lessios 1992). This is particularly true for tests which have a substantial power with respect to other alternative hypotheses, such as the X^2-g test, which is widely used in the earlier literature on *Mytilus edulis* (Table 3).

The X^2-2 test is also widely used (Table 3). This test uses the formula of Brown (1970), valid for only 2 alleles. Its use when more than 2 alleles are present is incorrect, as the asymptotic variance of F_{is} is $1/n_{(k-1)}$, k being the number of alleles, and not $1/n$ (Curie-Cohen 1982). As the latter quantity is larger than the former, the test using $1/n$ as the variance of F_{is} is more conservative. The other tests (X^2-h and f_T-test) are used in a minority of studies (Table 3).

In conclusion, the most widely used F_{is} estimator has a tendency to underestimate heterozygote deficiency. Most tests used in the *Mytilus edulis* literature either have a substantial power with respect to other alternative hypotheses to heterozygote deficiency (so that a significant p-value does not necessarily indicate a departure of HWE due to a heterozygote deficiency) or are not correctly used. In the latter situation (X^2-2 test),

reports of significant heterozygote deficiency are not to be reconsidered, as the incorrect test is more conservative. The existing literature concerning heterozygote deficiency in *M. edulis* (and probably in other marine molluscs as well) provides a conservative figure of the real phenomenon. This is confirmed by the large and significant heterozygote deficiencies present in all data sets analyzed with the same statistical tools (Tables 4 & 6 to 12).

Influence of the population genetic structure (Wahlund effect)

Although the random constitution of small and differentiated reproductive groups is an unlikely phenomenon to explain substantial heterozygote deficiency (e.g. Pudovkin & Zhivotovskii 1980), the various members of the *Mytilus edulis* complex are genetically differentiated. They do not have a continuous distribution, and they display numerous hybrid or contact zones when they meet. Some complex situations exist: in the British Isles at least 10 hybrid zones between *M. edulis* and *M. galloprovincialis* were identified (Skibinski & Beardmore 1979, Skibinski et al. 1983, Gosling 1984); on the French Atlantic coast there is also an intercalation of *M. edulis* and *M. galloprovincialis* and hybrids (Coustau et al. 1991, Koehn 1991); *M. trossulus* and *M. galloprovincialis* are both present along the Pacific coast of North America (plus *M. edulis* near Vancouver, see below), in a complex pattern depending on local ecological differences (Sarver & Foltz 1993), and these 2 taxa produce a contact zone (they do not hybridize extensively) when they meet (Rawson & Hillbish 1995); on the eastern North American coast *M. edulis* and *M. trossulus* are both found in a patchy pattern (Varvio et al. 1988, Koehn 1991, Bates & Innes 1995). For the latter geographic area, heterozygote deficiencies described before the discovery of this situation are best explained by a Wahlund effect, as suspected by Koehn et al. (1976).

The effect of recent extensive introduction of various members of the *Mytilus edulis* complex (through, e.g., commercial cultures or discharge of ballast water in transport ships) should not be ignored. For example, in the eastern and southern coast of Vancouver island, Canada, all populations of normally pure *M. trossulus* have been recently reported as 'contaminated' by *M. edulis* and *M. galloprovincialis* (Heath et al. 1995). Whether this phenomenon explains the heterozygote deficiency (Table 12) found in mussels sampled in 1971 and 1972 near Seattle, WA, USA, by Johnson & Utter (1973), or in other areas, remains to be investigated.

Recently, a secondary intergradation within *Mytilus galloprovincialis* has been described in Spain follow-

Table 12. Measures and tests of heterozygote deficiency for the Pacific American data set (6 populations). Weir & Cockerham (1984) and Robertson & Hill (1984) estimators of F_{is} are given for the *Aat* locus in each sample (respectively f_c and f_T). The estimate for all samples is indicated ('All'). Unbiased estimates of exact p-values of the *U*-score test (Markov chain of 50 000 steps for each locus in each population and of 20 000 steps for multi-population tests) are given. Bold characters indicate significant (p < 0.05) results

Sample	Aat		Test
	f_c	f_T	
Mukilteo	−0.030	+0.089	0.074
Picnic Point	+0.150	+0.134	**<2 × 10⁻⁵**
Alki Point	+0.489	+0.217	**0.011**
Seahurst	+0.277	+0.151	**0.005**
Saltwater	−0.036	−0.013	1
Dash Point	+0.097	+0.140	**0.019**
All	+0.162		**<5 × 10⁻⁵**

ing a large sampling effort around the Iberian Peninsula (Sanjuan et al. 1994, 1996, Quesada et al. 1995a, b). But the frequency of such phenomena in other geographic areas is unknown, and it is possible that other similar situations yet to be discovered could account for some reports of heterozygote deficiency.

It is also possible that the species complex *Mytilus edulis*, in addition to *M. edulis*, *M. galloprovincialis* and *M. trossulus*, contains other members not yet recognized, as suggested by sequences of 18S rDNA (Kenchington et al. 1995). If confirmed, the existence of additional sibling species could potentially explain many reports of heterozygote deficiency.

Another possibility is a temporal Wahlund effect, where differentiated cohorts are simultaneously sampled and analyzed. The existence of genetic differences between discrete settlement cohorts of *Mytilus edulis* has been found (Gosling & Wilkins 1985), so that this phenomenon could explain some reports of heterozygote deficiency whenever age classes could not be identified. A problem is that cohorts are not necessarily equivalent to size classes, as successive reproductive peaks may occur within a year for species such as *M. edulis* (Seed 1976, Gosling & Wilkins 1985). In addition, size and age classes are not exactly equivalent even for species showing only 1 annual peak of reproduction (David et al. 1995). In the few studies where age classes could be identified, some report heterozygote deficiency (Koehn & Gaffney 1984, Gosling & Wilkins 1985, Zouros et al. 1988) but others do not (Fevolden & Garner 1986). The absence of congruent ranking of $\hat{F}_{is}$ across populations for the various loci, for each study or overall (*Lap* excluded, Table 5), is consistent with a Wahlund effect (spatial or temporal).

Are the loci usually studied under some sort of direct or indirect selection explaining a frequent report of heterozygote deficiency?

At least for the *Lap* locus, the answer is clearly positive. The nature of the selection acting at the *Lap* locus has been the focus of biochemical, physiological and populational investigations (Koehn et al. 1980, Moore et al. 1980, Koehn & Immermann 1981, Koehn & Siebenaller 1981, Hilbish et al. 1982, Hilbish 1985, Hilbish & Koehn 1985, Beaumont et al. 1988, 1989), and selective processes can be suspected at least whenever larvae settle in a place where the salinity differs from where they originate. The $\hat{F}_{is}$ values at the *Lap* locus are large in all data sets (overall estimate for each data set between 0.08 and 0.16), and these deficits are significant in all data sets (Tables 6, 9 & 11), with the exception of the French set. Further information concerning water salinity experienced by

larvae before settlement is required to understand this pattern (Table 8).

For other loci, there are some hints in the literature that some sort of selection operates in some conditions (e.g. Beaumont et al. 1983, Mallet et al. 1985, Hilbish et al. 1994), although it is never demonstrated as clearly as for the *Lap* locus. In addition, the intricate geographical mosaic of the various members of the *Mytilus edulis* complex (Skibinski & Beardmore 1979, Skibinski et al. 1983, Gosling 1984, Varvio et al. 1988, Coustau et al. 1991, Koehn 1991, McDonald et al. 1991, Sarver & Foltz 1993, Sanjuan et al. 1994, Bates & Innes 1995) could introduce a bias, in particular in experiments conducted to show selection on enzymatic loci (Väinölä 1990).

No obvious heterogeneity of $\hat{F}_{is}$ could be detected, as the median of $\hat{F}_{is}$ for each locus does not vary significantly across data sets, and the median of $\hat{F}_{is}$ for each data set does not vary significantly across loci when *Lap* is excluded. This negative result does not prove that these loci are neutral, as the tests used probably have a low power with respect to alternative hypotheses of some variation of F_{is} across loci or across geographical areas. More specific tests should be constructed to possibly detect such variation, but this is beyond the scope of the present paper.

The absence of congruent effects across loci is consistent with the possibility that locus-specific factors are involved, and this situation is consistent with 1 or several spatially variable selective processes acting differently at several loci. This situation is also consistent with a mosaic distribution of differentiated populations or sub-species, with loci displaying different or similar gene frequencies across the distribution, thus generating a heterozygote deficiency through a Wahlund effect only for some loci.

Other possible factors

Some other factors potentially contributing to a heterozygote deficiency have not been thoroughly considered in this paper. They include, for example, allele misscoring, aneuploidy or the presence of null alleles. Allele misscoring cannot be ruled out as some loci are sometimes difficult to read, with a heterozygote being more often classified as a homozygote than the reverse (N. Pasteur pers. comm.). Such difficulty is probably variable across loci and laboratories (as all environmental conditions during electrophoresis cannot easily be replicated), and its consequence for the phenomenon of heterozygote deficiency remains to be investigated. It is worth noting that a possible misscoring of a heterozygote to a rare homozygote is unlikely to account for the heterozygote deficiency observed here,

as removing 2 different homozygotes and replacing them by 2 corresponding heterozygotes (thus keeping the allelic margin constant), for each test, would not have altered the conclusion, except for the locus *Est-D* in the French data set (details not shown). Aneuploidy has been documented in *Mytilus edulis* from a polluted area (Dixon 1982), but its significance in explaining heterozygote deficiency remains unclear. In addition, in the oyster *Crassostrea gigas*, where this problem has been thoroughly studied, aneuploidy has been found to be an unlikely explanation for this phenomenon (Zouros et al. 1996). The presence of null alleles at isozymic loci have been studied for *Mytilus*, and their low observed frequency in natural populations or during crosses cannot account for the level of heterozygote deficiency usually found (Mallet et al. 1985, Beaumont 1991, Gardner 1992).

CONCLUSION

Heterozygote deficiency is not clearly described in the literature, due to the variety of species studied, the various qualities of the F_{is} estimators used, and the pertinence of the different statistical tests applied. When several data sets are analyzed or re-analyzed using the same estimators and tests, the heterozygote deficiency still persists for the species complex *Mytilus edulis*. In this species complex, 2 factors are difficult to control: the spatial and temporal genetic differentiation and selection acting (directly or indirectly) on the marker loci. The latter is possible, but small variation of F_{is} across loci or geographical areas cannot be detected at present due to a lack of specific and powerful tests in this domain; hence the possibility that selection operates constantly to maintain the genetic identity of each taxon adapted to local conditions, despite high dispersal potential (Gardner 1996), cannot be excluded. Spatial and temporal genetic differentiation cannot be discounted due to the complex genetic structure found at various geographical scales and the continuous discovery of hybrid or contact zones, secondary intergradations or perhaps additional sibling species within the *M. edulis* species complex. This geographically or temporally complex pattern could explain some reports of heterozygote deficiency in the *M. edulis* species complex. In particular the temporal Wahlund effect has not been extensively studied, and its importance remains to be investigated in *Mytilus* as well as in other molluscs since the existence of genetically differentiated cohorts or complex spatial population dynamics has been reported in other marine molluscs (e.g. Johnson & Black 1984, Borsa et al. 1991, Lewis & Thorpe 1994, David et al. 1996).

Due to the intricate situation encountered with *Mytilus edulis*, detailed studies similar to the present study are required for other species before any general conclusion can be drawn. Interesting results should come out from the comparison of allozyme loci and other markers such as anonymous DNA fragments or anonymous genes, but it is too early to draw general conclusions from the few recent papers published in this area (e. g. Pogson & Zouros 1994, Hare et al. 1996), especially as these have opposing conclusions.

As heterozygote deficiency is also present in other species such as self-incompatible or essentially outbreeding plants (Levin 1978, Cuguen et al. 1988), it is likely that there is no unique explanation for the problem of heterozygote deficiency in marine bivalves (see e.g. Gaffney et al. 1990) and in other organisms, and it is not excluded that species-specific, locus-specific or population-specific explanations are to be sought.

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