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Multiple-locus heterozygosity, physiology and growth at two different stages in the life cycle of the Chilean oyster *Ostrea chilensis*

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ABSTRACT: A random sample of 150 individuals of a laboratory-produced cohort of *Ostrea chilensis* Philippi, 1845 was taken at 10 and 36 mo of age to estimate physiological variables and individual heterozygosity using 4 loci (*Lap*, *Pgi*, *Pgm* and *Ca*). Juveniles of 10 mo of age showed a mean D value of 0.134 ($p > 0.05$) and a positive correlation between oyster size and multiple-locus heterozygosity (MLH) ($p < 0.05$). Also, there was a positive correlation ($p < 0.05$) between ingestion rate, absorption rate and MLH. A negative correlation between excretion rate ($p > 0.05$), oxygen consumption rate ($p < 0.05$) and MLH was found. The K_2 value (standardized net growth efficiency) was positively correlated ($p < 0.05$) with MLH. At 36 mo a heterozygote deficiency was present with a mean value $D = -0.431$ ($p < 0.05$). No relationship between standard dry weight and MLH and also a negative correlation between the scope for growth and MLH were found. The oxygen consumption and excretion rates also showed an increase in large size individuals. The slopes for filtration and excretion rates against MLH were negative and not statistically significant. However, ingestion and absorption rates showed significant ($p < 0.05$) decrease with an increase in heterozygosity. The results seem to indicate that within sexually immature individuals of *O. chilensis*, a positive correlation between growth rate and MLH can be found, while in adults the higher energy allocation for reproduction precludes the detection of this relationship.

KEY WORDS: Growth · Heterozygosity · *Ostrea chilensis* Oyster · Physiology

INTRODUCTION

The observation that multiple-locus allozyme heterozygosity (MLH) correlates positively and significantly with growth rate and metabolic efficiency has been made for many organisms (Mitton & Grant 1984). Among the marine invertebrates, higher age-specific growth rate has been demonstrated with increasing mean heterozygosity at electrophoretically detectable loci in natural populations of oysters (Singh & Zouros 1978, Zouros et al. 1980, Fujio 1982, Koehn & Shumway 1982), mussels (Koehn & Gaffney 1984, Diehl et al. 1986, Rodhouse et al. 1986, Gentili & Beaumont 1988), clams (Garton et al. 1984, Koehn et al. 1988) and snails (Garton 1984). Demonstration of a correlation between heterozygosity and growth rate depends on genetic structure of the population (Gaffney & Scott 1984), accuracy of age determination, and life stage sampled. When the energy is allocated largely to somatic growth rather than to reproduction, the correlation can disappear (Rodhouse et al. 1986).

Within the context of a balanced energy budget (Thompson & Bayne 1974), scope for growth represents the energy available for growth and reproduction, expressed as the difference between absorbed energy and energy lost through metabolic processes. According to this, the physiological basis by which more heterozygous individuals achieve higher average growth rates may include increasing metabolic efficiency (i.e. reduction of metabolic costs per unit

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weight) and/or increasing energy absorption (i.e. increased feeding rate or absorption efficiency or both). Several studies relate heterozygosity to metabolic parameters. They include standardized oxygen uptake during starvation in *Crassostrea virginica* (Koehn & Shumway 1982) and *Mytilus edulis* (Diehl et al. 1986), weight loss during starvation in *C. virginica* (Rodhouse & Gaffney 1984), protein turnover in *M. edulis* (Hawkins et al. 1986) and density-related stress in *M. edulis* (Gentili & Beaumont 1988). A more complete study carried out in *Mulinia lateralis* included the measurement of several metabolic parameters and showed that 'scope for activity' was positively correlated to heterozygosity (Garton et al. 1984). We describe here an experiment in which size, physiological variables (energy budget) and MLH were measured for individual *Ostrea chilensis* Philippi, 1845 from a cohort produced in the laboratory and grown in the field under uniform low-density conditions until 10 and 36 mo of age.

MATERIALS AND METHODS

Oysters. A cohort of *Ostrea chilensis*, collected during December 1987 from a natural spatfall from the wild population of *O. chilensis* at Quempillén River Estuary in Chile (45° 52' S, 73° 46' W), was used as parental stock. In September 1990, after 3 yr of growth under uniform conditions at Hueihue location (Chile) (41° 58' S, 73° 30' W), 800 randomly chosen oysters were taken as brood stock to produce a cohort in the laboratory. By using mass spawning it is not possible to determine how many individuals contribute genes to the offspring obtained; however, some estimation of the female contribution was made by the number in each brood of eyed larvae. The brooding oyster *O. chilensis* presents an average fecundity of 60000 larvae per season (Toro & Chaparro 1990). Thus, from the amount of larvae released, more than 8.2×10^6, we assumed that at least 130 female had contributed larvae. This estimation of the female contribution may be an underestimation because in this species some of the eyed larvae released will set in as few as 5 min (DiSalvo et al. 1983). The male contribution in this experiment can be assumed (about 300 to 400 males) by the percentage of males found in the field site during the spawning season that fluctuates between 45 and 60% of the population. The brood oysters were placed in a fiberglass tank and warmed from an ambient water temperature (around 12°C) to 18°C over a 4 wk period. Salinity during conditioning was 27 to 28 ppt, corresponding to the average salinity for the months of September to November in the estuary. The salinity variation in the Quempillén Estuary throughout the year ranges between 14 and 32 ppt with an annual average of 26 ppt, while the temperature fluctuates between 9 and 22°C with an annual average of 13°C. The water was filtered through a 5 µm filter and before heating, passed through an ultraviolet (UV) sterilizing unit. The water was changed daily and a mixture of *Isochrysis galbana*, Clone T-iso, and *Chaetoceros gracilis* Schutt was added continuously to a final concentration of 50 cell μl^{-1} After 5 wk of conditioning, clean plastic plates (15 × 15 cm) were placed in the tank. The larval release and settlement occurred within 24 h. Each collector was labeled and transferred to a common rearing tank for 5 wk. Individually tagged juveniles were grown in the field under uniform low-density conditions (150 oysters m^{-2}) following the procedures of Toro & Varela (1988). Samples of 150 randomly chosen oysters were collected at 10 mo of age (live weight: 0.104 to 1.570 g; shell height: 8 to 23.8 mm) and at 36 mo of age (just after the spawning season; live weight: 8.65 to 47.2 g; shell height: 34.6 to 66.9 mm) in order to make direct, individual measurements of growth, energy budget and MLH.

Energy budget. For each individual oyster, after a period of 3 d of acclimatation in the laboratory, the following physiological variables were estimated or calculated, with 3 replicates each, in order to describe the energy budget at 13°C and 28 ppt salinity.

Filtration rate: This rate was determined indirectly by quantifying the rate of decrease in algal cell density in the experimental medium maintained in darkness (10 and 2 l for each oyster of 36 and 10 mo of age respectively). Initial algal concentration was 15×10^6 cells of *Isochrysis galbana* per liter (Navarro & Winter 1982; Vergara et al. 1992). Every 2 h, algal concentration was measured by Coulter counter (ELZONE 180XY) and the initial concentration restored.

Organic ingestion rate: The amount of organic food ingested per unit time (mg organic matter d^{-1}) was calculated as the product of filtration rate (no pseudofaeces were observed) and organic dry weight of *Isochrysis galbana* (Winter et al. 1984).

Assimilation efficiency (%): This was determined according to Conover (1966). Faeces were collected, washed and dried immediately at the end of each filtration rate experiment in order to avoid decomposition.

Absorption rate: The product of ingestion rate and assimilation efficiency was expressed in joules per day (J d^{-1}) (1 mg of organic material of *Isochrysis galbana* = 18.75 J; Whyte 1987).

Excretion rate: This was determined by measuring g ammonia nitrogen produced per oyster per hour (Solórzano 1969). Juvenile oysters were incubated in 100 ml and adult oysters in 300 ml of 0.45 µm filtered seawater for 5 h. Controls were filtered seawater from

the same batch, without oysters, incubated at the same time. Amount of ammonia nitrogen was transformed to energy (1 mg NH_4-N = 24.8 J) using the coefficient of Elliot & Davison (1975).

Respiration rate: This was measured in the apparatus described by Rios (1979). The oysters were starved for 3 d in filtered seawater before the experiment (standard or basal metabolism). Values for oxygen consumption were expressed in ml O_2 h^{-1} and transformed to energy using the coefficient of Thompson & Bayne (1974) (1 ml O_2 = 19.9 J).

Scope for growth: This was calculated as the difference between absorbed energy and energy loss to excretion and respiration, expressed in J d^{-1} (Warren & Davis 1967).

Net growth efficiency (K_2): The efficiency with which organic material is transformed into body tissue was the dividend of scope for growth divided by absorbed energy (Winter et al. 1984).

Electrophoresis. After physiological evaluation, oysters were placed in liquid nitrogen until electrophoresis. Starch gel electrophoresis was used to score loci for leucine aminopeptidase (*Lap*, EC 3.4.1.1), glucose phosphate isomerase (*Gpi*, EC 5.3.1.9.), carbonic anhydrase (*Ca*, EC 4.2.1.1) and phosphoglucomutase (*Pgm*, EC 2.5.7.1.) following Shaw & Prasad (1970), Selander et al. (1971) and Ahmad et al. (1977) These 4 polymorphic loci were chosen because they have been already used in earlier studies on *Ostrea chilensis* (Toro & Aguila 1995).

For each locus, observed genotypic distributions were compared with Hardy-Weinberg expectations (*G*-test for goodness of fit) (Sokal & Rohlf 1981). Heterozygote deficiencies were expressed as $D = (H_o - H_e)/H_e$, where H_o is the observed frequency of heterozygotes and H_e the expected frequency.

Observed MLH distributions were determined by counting the number of individuals heterozygous for 0, 1, ..., k loci. Because sample sizes varied among MLH distributions, weighted least squares regressions were employed for physiological variables on MLH.

Statistics and weight standardization. Weight-specific rate functions were calculated in order to eliminate the differential weight of oysters on each physiological rate and quantify only the genetic effect (Packard & Boardman 1988). The weight standardization was carried out using analysis of covariance (ANCOVAR) with dry tissue weight as the covariate, physiological rate as the dependent variable and the heterozygosity classes as the treatments. The appropriate regression coefficients were then used to adjust individual physiological rates.

RESULTS

The percentage mortality of experimental oysters, between settlement and 10 mo of age was 6% and between the ages of 10 and 36 mo it was 27%. The 4 enzyme loci studied were in Hardy-Weinberg equilibrium in the 10 mo old juveniles (Table 1). However, in the 36 mo old oysters of the same cohort *Lap*, *Pgm* and *Ca* differed significantly from Hardy-Weinberg expectations with a deficiency of heterozygotes (Table 2).

Table 1. *Ostrea chilensis*. Observed number of genotypes (*Lap, Pgi, Pgm, Ca*) in the cohort at 10 mo. Expected number for Hardy-Weinberg and χ^2 is also shown. Numbers in parentheses correspond to observed and expected genotypes. ns: not significant; N = 110

Genotype		Observed	Expected	χ^2	p
Lap	100/100	0.516 (56)	(56.7)		
	100/90	0.405 (46)	(44.5)	0.12	ns
	90/90	0.079 (8)	(8.7)		
Pgi	100/100	0.478 (50)	(52.5)		
	100/90	0.426 (52)	(47.0)	1.255	ns
	90/90	0.096 (8)	(10.5)		
Pgm	100/100	0.515 (60)	(56.7)		
	100/95	0.405 (38)	(44.5)	2.364	ns
	95/95	0.080 (12)	(8.7)		
Ca	100/100	0.226 (22)	(26.5)		
	200/100	0.290 (32)	(27.5)		
	150/100	0.290 (32)	(27.5)	5.204	ns
	200/200	0.065 (8)	(7.1)		
	200/150	0.065 (8)	(14.3)		
	150/150	0.065 (8)	(7.1)		

Table 2. *Ostrea chilensis*. Observed number of genotypes (*Lap, Pgi, Pgm, Ca*) in the cohort at 36 mo. Expected number for Hardy-Weinberg and χ^2 is also shown. Numbers in parentheses correspond to observed and expected genotypes. ns: not significant; N = 142; $^*p < 0.05$

Genotype		Observed	Expected	χ^2	p
Lap	100/100	0.438 (70)	(62)		
	100/90	0.448 (48)	(64)		
	90/90	0.114 (24)	(16)	8.50	*
Pgi	100/100	0.690 (100)	(98)		
	100/90	0.281 (36)	(40)	1.35	ns
	90/90	0.029 (6)	(4)		
Pgm	100/100	0.702 (108)	(100)		
	100/95	0.272 (22)	(38)	26.171	*
	95/95	0.026 (12)	(4)		
Ca	100/100	0.338 (48)	(33)		
	200/100	0.099 (14)	(38)		
	150/100	0.197 (28)	(33)	40.788	*
	200/200	0.169 (24)	(11)		
	200/150	0.113 (16)	(19)		
	150/150	0.084 (12)	(8)		

Table 3. *Ostrea chilensis*. Average values for *D* in each locus (*Lap*, *Pgi*, *Pgm*, *Ca*) and weight class category and their correlations (r) with the oyster log dry weight. $^{*}p < 0.05$; $^{**}p < 0.01$

10 months					
Locus	Oyster dry weight class (g)				r
	0–0.013	0.014–0.026	0.027–0.039	>0.040	
Lap	−0.659	0.181	0.310	0.384	0.837**
Pgi	−0.206	0.005	0.390	0.396	0.613**
Pgm	−0.321	0.211	0.198	0.486	0.784**
Ca	−0.127	0.264	0.231	0.415	0.921**
Mean *D*	−0.328	0.165	0.282	0.420	
36 months					
Locus	Oyster dry weight class (g)				r
	0–0.360	0.361–0.720	0.721–1.080	>1.081	
Lap	0.200	0.150	0.074	0.060	−0.940**
Pgi	0.124	0.119	0.217	−0.213	−0.579**
Pgm	0.054	0.096	0.099	0.083	0.358*
Ca	0.353	−0.430	−0.645	−0.357	−0.292
Mean *D*	0.182	−0.016	−0.063	−0.106	

The relative viabilities (Alvarez et al. 1989) between 10 and 36 mo for heterozygotes were 0.347 (*Pgm*), 0.364 (*Ca*), 0.710 (*Lap*) and 0.379 (*Pgi*).

The 2 age classes were divided into 4 subgroups each, based on individual dry weight. Table 3 shows the distributions of *D* for each locus and averages over loci in 4 weight groups. From the data it can be seen that the deficiency of heterozygotes is not equally distributed over weight and age classes. The higher weight classes are not deficient in heterozygotes in 10 mo old oysters, showing a positive value of *D* and a negative value of *D* in the smallest group of the cohort, while the opposite can be observed in 36 mo old oysters at 2 loci.

A positive and significant relation between standard dry weight and MLH was found for the 10 mo old juveniles of the cohort (Fig. 1a). After adjustment for the regressions of energy budget parameters on estimated somatic dry weight, there was a significant positive relationship between scope for growth and MLH among 10 mo old oysters (Table 4). Regressions of ingestion rate, absorption rate, net growth efficiency (K_2) and total energy gains against MLH were each positive and significant (Table 4). Respiration rate (weight-specific metabolic costs) showed a negative and significant relationship with MLH, while excretion rate and total energy losses showed a negative trend to heterozygosity, but not significantly (Table 4). MLH explained 17.9% of the variation in weight-corrected metabolic energy gains and 8% of the variation in weight-specific metabolic costs (Table 4, Fig. 1).

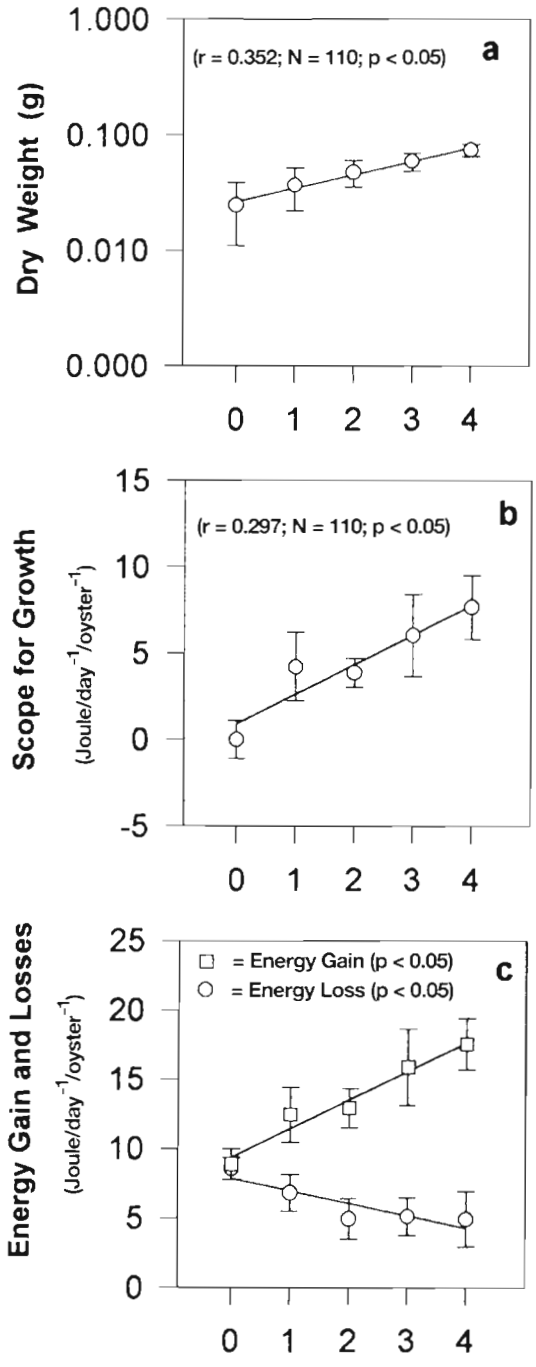

Fig. 1. *Ostrea chilensis*. Regressions of (a) dry weight, (b) scope for growth and (c) energy gain and losses on multiple-locus heterozygosity in 10 mo old oysters. Values are means ± SE. See also Table 4

The regressions after adjustment of energy budget parameters on estimated somatic dry weight presented a significant negative relationship between scope for growth and MLH among 36 mo old oysters (Table 5). Regressions of ingestion rate, absorption rate, net

Table 4. *Ostrea chilensis*. Analyses of weight-specific energy budget components on multiple-locus heterozygosity (MLH) in 10 mo old oysters. OM: organic matter; ns: not significant; N = 110; $^{*}p < 0.05$

Parameter	Equation	r	p
Filtration rate (l h^{-1} $oyster^{-1}$)	0.067 - 0.014(MLH)	0.121	ns
Organic ingestion rate (mg OM d^{-1} $oyster^{-1}$)	0.615 + 0.192(MLH)	0.296	*
Absorption rate (mg OM d^{-1} $oyster^{-1}$)	0.712 + 0.144(MLH)	0.282	*
Excretion rate (g NH_4-N d^{-1} $oyster^{-1}$)	36.876 - 0.521(MLH)	0.073	ns
Respiration rate (ml O_2 d^{-1} $oyster^{-1}$)	0.456 - 0.085(MLH)	0.391	*
Scope for growth (J d^{-1} $oyster^{-1}$)	2.748 + 0.842(MLH)	0.297	*
Net growth efficiency (K_2)	0.211 + 0.162(MLH)	0.469	*
Total energy gains (J d^{-1} $oyster^{-1}$)	10.537 + 1.269(MLH)	0.423	*
Total energy losses (J d^{-1} $oyster^{-1}$)	6.741 - 0.564(MLH)	0.290	ns

Table 5. *Ostrea chilensis*. Analyses of weight-specific energy budget components on multiple-locus heterozygosity (MLH) in 36 mo old oysters. OM: organic matter; ns: not significant; N = 142; $^{*}p < 0.05$; $^{**}p < 0.01$

Parameter	Equation	r	p
Filtration rate (l h^{-1} $oyster^{-1}$)	1.427 - 0.043(MLH)	0.061	ns
Organic ingestion rate (mg OM d^{-1} $oyster^{-1}$)	37.242 - 4.434(MLH)	0.283	*
Absorption rate (mg OM d^{-1} $oyster^{-1}$)	32.365 - 4.261(MLH)	0.254	*
Excretion rate (g NH_4-N d^{-1} $oyster^{-1}$)	232.798 +13.512(MLH)	0.116	ns
Respiration rate (ml O_2 d^{-1} $oyster^{-1}$)	4.706 + 0.788(MLH)	0.275	*
Scope for growth (J d^{-1} $oyster^{-1}$)	557.362 - 94.259(MLH)	0.323	*
Net growth efficiency (K_2)	0.831 - 0.328(MLH)	0.393	**
Total energy gains (J d^{-1} $oyster^{-1}$)	433.502 -52.872(MLH)	0.247	*
Total energy losses (J d^{-1} $oyster^{-1}$)	111.634 +11.641(MLH)	0.223	*

growth efficiency (K_2) and total energy gains, against MLH were each negative and significant (Table 5). Respiration rate and total energy losses (weight-specific metabolic costs) each showed a positive and significant relationship with MLH, while excretion rate showed a negative trend to heterozygosity, but not significantly (Table 5). There was no relationship between standard dry weight and MLH (Fig. 2a).

DISCUSSION

Deficiency of heterozygotes in marine bivalves is a well-known phenomenon. Laboratory studies (Mallet et al. 1985, Gaffney et al. 1990) and studies using wild populations (Gosling & Wilkins 1985, Gosling & McGrath 1990) in *Mytilus edulis* have found this deficiency. In oysters, several studies have reported this phenomenon (Singh & Zouros 1978, Zouros et al. 1980 for *Crassostrea virginica*, Guiñez & Gallegillos 1985 for *Ostrea chilensis*). The results in the present study, however, are not in accord with those reported by Singh (1982) where the average observed heterozygosity increased and average *D* (over loci) decreased with increasing age in wild cohorts of *C. virginica*. In laboratory studies with mussels an overall deficiency of heterozygotes was found at the juvenile stage but not at the spat stage (Beaumont 1991), suggesting selection against heterozygotes as the most probable cause. Such selection would also explain the results found in the laboratory-produced cohort of *O. chilensis* in this study. Alvarez et al. (1989), working with *O. edulis*, report a strong negative correlation between heterozygosity and viability with a mean viability of heterozygote oysters for 1, 2 and 3 loci of 0.51 ± 0.12, between 18 and 30 mo of age, a value that is close to the one found in the present study (0.46 ± 0.17).

A form of a balancing selection proposed by Singh (1982) could explain the increasing deficiency of heterozygotes with age, by genotype-dependent mortality after settlement. *Ostrea chilensis* broods its larvae within the mantle cavity until the eyed larvae stage, with a very short planktonic period that ranges between 5 min and 48 h (DiSalvo et al. 1983). The fast growing heterozygous spat will have higher food (phytoplankton) requirements after their settlement during the last week of December (summer), when the number of cells l^{-1} of natural phytoplankton shows a marked decrease in the Quempillén Estuary (Toro 1985, Senn 1993). These heterozygous individuals will then face starvation and mortality, producing the heterozygote deficiency in the later stages of the life cycle of the *O. chilensis* cohort (Toro & Vergara 1995). Another alternative hypothesis to explain the increasing

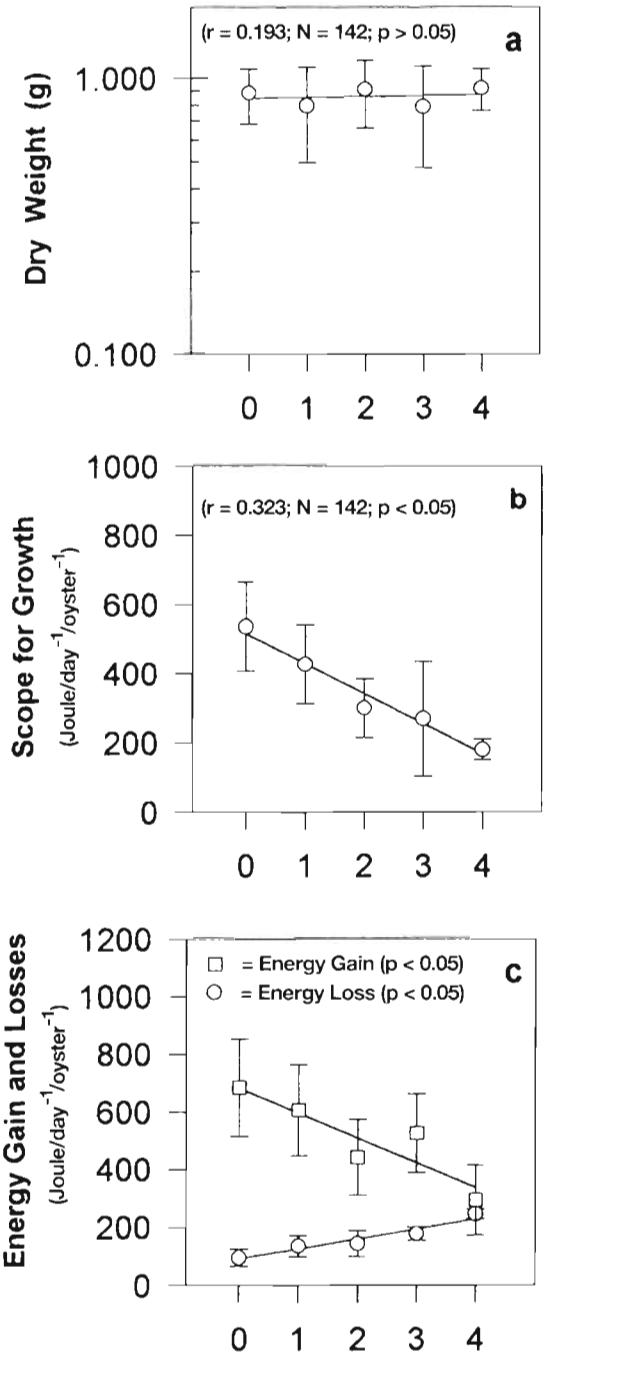

Fig. 2. *Ostrea chilensis*. Regressions of (a) dry weight, (b) scope for growth and (c) energy gain and losses on multiple-locus heterozygosity in 36 mo old oysters. Values are means ± SE. See also Table 5

deficiency of heterozygotes could be that multi-locus heterozygotes are victims of their own efficiencies at later stages of their life cycle. Reproduction is a very energy demanding process and many oysters succumb to disease and/or are metabolically spent after gametogenesis (Chaparro 1995). It could be possible that these very efficient oysters overproduce gametes and then attempt to brood more offspring than they can care for and still remain healthy and/or continue growing.

The deficiency of heterozygotes was not evenly distributed among the weight classes within each cohort (Table 3), with negative values for D in the smallest group and positive values of D in the heaviest group for the 10 mo old cohort. The opposite was found in the 36 mo old cohort. This was reflected in the energy budget correlations, showing that the lower scope for growth was found within the more heterozygote deficient oysters (Figs. 1c & 2c).

A lack of a strong single-locus effect between metabolic costs and heterozygosity does indicate that the influence of heterozygosity is additive across loci in the 10 mo old oysters, resulting in heterosis for genotype-dependent growth rate (Fig. 3). This heterosis for growth rate has been observed in other mollusc species (Singh & Zouros 1978, Garton et al. 1984, Koehn & Gaffney 1984).

These results for the 10 mo old oysters are in accordance with previous reports on the relationship between MLH and growth rate (Singh & Zouros 1978, Zouros et al. 1980, Fujio 1982, Koehn & Shumway 1982, Garton 1984, Garton et al. 1984, Koehn & Gaffney 1984, Diehl et al. 1986, Rodhouse et al. 1986, Gentili & Beaumont 1988, Koehn et al. 1988).

These results are in accordance with those carried out in older (>2 yr) individuals by Foltz & Zouros (1984) in *Placopecten magellanicus* and by Beaumont et al. (1985) in *Pecten maximus*. These authors failed to show a growth rate/heterozygosity correlation. The reason suggested by Rodhouse et al. (1986) is the increasing allocation of energy to gamete production in these older individuals. In bivalves the somatic growth exceeds gamete production in early life, reaches a peak in intermediate age individuals and then declines in later life, while gamete production is null or low in early stages of the life cycle, but increasing throughout the life of the bivalve (Thompson 1984). The relationship shown in Fig. 2a corresponds to standard somatic dry weight after spawning (during the brooding period), thus there were no gametes included in the weight. As stated before, *Ostrea chilensis* broods its larvae for a period of 5 to 8 wk, requiring higher energy costs during the brooding period that may contribute to weight loss (Chaparro et al. 1993). These higher energy costs can be reflected in the negative scope for growth shown in Fig. 2b and also the higher oxygen consumption reflected in the total energy losses in Fig. 2c.

Although this cohort of oysters was produced in the laboratory using mass spawning, we calculated that at least 130 females had contributed larvae. Thus, this

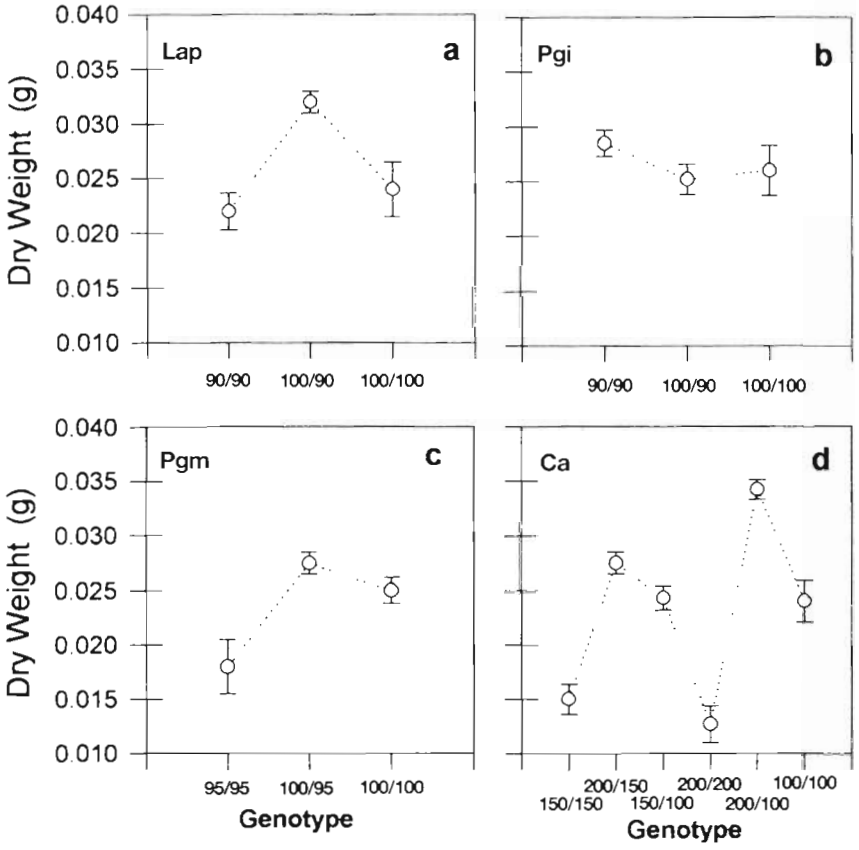

Fig. 3. *Ostrea chilensis*. Mean dry weight (± standard error) of homozygous and heterozygous genotypes for each locus in 10 mo old oysters. (a) locus *Lap*; (b) locus *Pgi*; (c) locus *Pgm* and (d) locus *Ca*

cohort cannot be treated as a product of restricted matings to explain the lack of a positive heterozygosity/growth correlation in the presence of a significant deficiency of heterozygotes (Beaumont 1991).

Our findings are consistent with other reports that have demonstrated energetic advantages of more heterozygous individuals. In sexually immature juveniles of *Ostrea chilensis* produced by mass spawning in the laboratory, a positive correlation between growth rate and MLH can be found, while in adults the higher energy allocation for reproduction precludes the detection of this positive relationship with growth rate.

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