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Note

Heritability estimates of larval shell length in the Chilean blue mussel *Mytilus chilensis*, under different food densities

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Abstract

Heritability of larval growth rate in diferent food environments and genotype-food environment interaction (during larval growth) are estimated in *Mytilus chilensis*, based on a nested design, involving 24 full-sib families (8 males each mated to 3 separated females) grown under three different algal concentrations. The heritability estimates for larval shell length ranged between 0.2 ± 0.1 and 0.9 ± 0.3 and showed a decreased towards the low food treatment; but the values suggest that selection would be effective. However, since there is a significant genotype-food interaction, selection should be done in each environment in which the animals are expected to perform. The significant interaction term ($p < 0.001$) associated with a significant genetic variability for larval growth suggest the presence of genes in the population whose expression depends upon local food conditions.

Keywords: *Mytilus chilensis*, heritability, larval growth, Chile.

Bivalve aquaculture has enormous potential in southern Chile mainly because the numerous sheltered bays and estuaries along its coastline are free from industrial pollution. Fertilizers and pesticides are rarely applied in local agriculture, and domestic pollution, owing to the very low human population densities in southern coastal areas, is small (Winter *et al.*, 1984; Toro and Chaparro, 1990). The culture of *Mytilus chilensis* (Hupé, 1854) in Chile has been increasing rapidly in the past years (Winter *et al.*, 1984; Navarro and Gutierrez, 1990; SERNAP, 1994); because of this potential, estimates of genetic variation (*i.e.* heritability) are not only important from the purely ecological point of view, but also for practical management purposes (Mallet *et al.*, 1987). The type of selection scheme that is appropriate depends upon the relative magnitude of the variation of that trait due to differences in genotype and in environmental factors (Falconer, 1989). Several workers have reported on heritability estimates based on half-sib correlation analysis for the blue mussel *Mytilus edulis*. For example, Innes and Haley (1977) reported a heritability estimate, based on a salinity tolerance experiment, a value of $h^2 = 0.16$ for *M. edulis* larvae (Innes and Haley, 1977). Low heritability ($h^2_{\text{sire}} = 0.11 \pm 0.02$, $h^2_{\text{dam}} = 0.19 \pm 0.04$) have been reported for the shell length of *M. edulis* larvae (Mallet *et al.*, 1986), however, Stromgren and Nielsen (1989) reported very high heritability estimates ($h^2 = 0.5 \pm 0.2 - 0.9 \pm 0.3$) in *M. edulis* based on a nested design involving 27 full-sib families.

Based on 8 enzyme loci, McDonald *et al.* (1991) and Koehn (1991) consider *M. chilensis* to be synonymous with *M. edulis*. However, according to Sarver and Foltz (1993), any debate about species level taxonomy is largely dependent on the species definition which is used. Thus, in order to clarify the dispute about taxonomy of species in the genus *Mytilus*, additional enzyme loci, and other characters such as mitochondrial DNA, and samples from additional locations, will be needed. Currently a PCR-based nuclear marker (Heath *et al.*, 1995) is being used to discriminate between *M. edulis* and *M. trossulus* in eastern Newfoundland and *M. chilensis* samples from southern Chile.

In this study, we consider the influences of food concentration and family on rates of larval growth. To date, there have been no reports on growth and survival of hatchery produced seed of *M. chilensis*. The present study represents the first attempt to estimate heritability values for growth rate in larval stages in this Mytilus species from the southern hemisphere.

MATERIAL AND METHODS

Mussels and gametes

Adult mussels (N = 115; 56.1–58.4 mm) (*Mytilus chilensis* Hupé, 1854) were collected from a mussel farm located in Yaldad Bay, Chiloé Island (43° 08′ S; 73° 44′ W), southern Chile during late December (spawning season). The mussels were kept out of water for about 4-5 h before attempting spawning by thermal shock. They were rinsed in clean seawater and placed in a 20-litre plastic tray with filtered (1-μm) and U.V. treated sea water (FSW) at 18°C. They were then continuously monitored and transferred to individual beakers once they began spawning. The eggs and sperm were held about for 1 h at room temperature (18°C) before use, to help synchronize egg stage (Scarpa and Allen, 1992).

Experimental design and larval cultures

Heritability of larval growth rates in different food environments (during larval growth) was estimated in *M. chilensis* grown under three different algal concentrations with three replicates, based on a balanced nested mating design (Becker, 1992). From the parental stock, 24 females and 8 males were chosen at random. Each male was mated to 3 females, involving 24 full-sib families (progeny sharing a common mother and father) and 8 half-sib families (progeny sharing a father but having different mothers) (Falconer, 1981). The embryos from each family were subdivided into 3 groups and each group further subdivided (replicates) and placed into three 10-litre plastic buckets containing 8 litres of fresh sea water (FSW) 16 ± 1°C, at a density of 100 individuals per ml. After 24 h (D-stage larvae) the density was adjusted to 5 larvae per ml. Three different cell concentrations of the micro algae *Isochrysis galbana* (T-iso) were used as treatments: 6 250; 25 000 and 100 000 cells/ml were the low, medium and high food concentrations respectively (Toro and Paredes, 1996). Every other day the water in each beaker was passed through a 45-micron "nitex" screen to retain the larvae. Each beaker was rinsed with fresh water followed by seawater. The larvae were then resuspended in FSW, Algal food was added daily at the desired cell concentration.

Sampling and data analysis

Samples for analyzing larval growth were taken from the beakers at 6, 12, and 20 days after fertilization. At each time the cultures were well mixed and a sub-sample of 8 ml (approx. 30-40 larvae from each replicate of each full-sub family) was taken from each beaker. Larval samples were preserved in 10% buffered formalin and stored at 4°C until analysis. Length (m) of larvae was measured, using a Zeiss IM35 inverted microscope.

The heritability values and their standard errors were calculated for each nutrition level, following Becker (1992). The genotype-food interactions were estimated by a nested-factorial analysis of variance, using the SYSTAT 5.1 statistical package (Wilkinson, 1991). In the model statement:

$Y_{ijklm} = \mu + \text{Sire}_{(i)} + \text{Dam}_{(j/i)} + \text{Food}_{(k)} + \text{Food}_{(k)} \times \text{Sire}_{(i)} + \text{Food}_{(k)} \times \text{Dam}_{(j/i)} + R_{(l/k)} + E_{(ijklm)}$, log-length was considered the dependent variables (Y); μ was the population mean; and the dummy variables were $\text{Sire}_{(i)}$, $\text{Dam}_{(j)}$, and $\text{Food}_{(k)}$ (where $k = 1, 2$ and, 3 were the low, medium and high food densities respectively; $i = 1, 2, ..., 8$ were the males and $j = 1, 2$, and 3 were the females nested within each male) were considered as independent variables; $E_{(ijklm)}$ was the error term.

RESULTS AND DISCUSSION

Significant differences ($p < 0.001$) were found in the size of the larvae between food levels where larvae from the 24 full-sib families were used. No significant differences between replicate beakers at the same food level were detected ($p > 0.05$) (Table 1). The average size of the larvae were significantly larger ($p < 0.05$), as food concentration increased up to 100000 cells/ml (Table 2). At the low food level there is considerable variability between individuals of the same family. This is reflected in the lower heritability estimates obtained. Since there was a significant genotype-food interaction (Table 1), any selection for growth rate should be done within each environment in which the animals are expected to perform. The significant interaction term ($p < 0.001$) associated with a significant genetic variability for

Table 1. – Analysis of variance used to test for significant variation in shell length in 20 days-old larvae.

Source	Degree of freedom	Sum of squares	F-ratio
Among sires	7	422 621	61.5*
Among dams within sires	16	274 013	17.4*
Among food rations	2	29 743	15.1*
Sire X food ration	14	119 093	8.7*
Dam with sire X food ration	46	182 409	4.0*
Replicates within food ration	1	299	0.3NS
Error	4 955	4 903 022	

* significant $p<0.001$; NS non significant.

larval growth suggest that selection for growth rate at different food levels is not selection for the same trait, in other words, different traits are under selection in each treatment.

The heritability estimates were relatively high and decreased with food concentration (Table 2). According to Falconer (1989), the heritability is a property not only of the trait, but also of the population, of the environment in which the individuals are raised and by how the phenotype is measured. Any change in the above components of variance will thus affect the heritability estimates. In the present study, larvae culture conditions affect significantly the heritability values for growth rate, reducing the heritability at lower food rations. However, the heritability values within the highest food environment suggest that selection would be effective (Table 2). The "dam" estimates (h_d^2) are significant and all cases slightly higher than the "sire" estimates, indicating the presence of some maternal and dominance variance effects.

Heritability estimates in several bivalve larval stages have been reported. For *Crassostrea gigas* (Lannan, 1972), estimates of $h^2 = 0.31$ have been obtained for larval survival, and for various spat traits at 12 months, h^2 ranges between 0.31 and 1.17. Another study (Longwell and Stiles, 1973) reports an h^2 of 0.24 for the growth rate of *Crassostrea virginica* (Gmelin) larvae at 14 days. Several other studies also describe heritabilities for larval growth rates in *C. virginica* by analyzing full and half-sib families; these estimates ranged between 0.25 and 0.71 (Haley *et al.*, 1975; Newkirk *et al.*, 1977; Losee, 1978). More recently a study based on a nested design, reported heritabilities for larval growth rate in *M. edulis* ranging between $h^2 = 0.5 \pm 0.2$ and 0.9 ± 0.3 (Stromgren and Nielsen, 1989). These estimates agree with the results obtained in the present study and it seems that a large portion of these traits show additive genetic variation.

Several studies on bivalves carried out in the laboratory have shown that ther is little or no correlation between shell growth of larvae and juveniles (Stromgren and Nielsen, 1989; Hilbish *et al.*, 1993), but it is not known if there is a lack of correlation between larval and shell growth in natural populations. However, we expect that heritability values between these two life cycle stages could be disconnected; studies have shown that heterozygotes have lower viability than homozygotes during the larval stage (Mallet and Haley, 1983; Mallet *et al.*, 1985) thus, changing the allelic frequencies.

Heritability values of 0.20 or larger indicate that genetic progress can easily be achieved through the application of selective breeding programs (Newkirk *et al.*, 1977; Falconer, 1989). In the ligth of the difficulty in selecting individuals which grew fast, as larvae to breed from, the application of family selection instead of individual selection was the option used in the present study. Using the most conservative heritability estimates (sire component) obtained for larval growth in *M. chilensis*, and applying a selection intensity of 1.755 that is equivalent to select the higher

Table 2. – *Mytilus chilensis*. Heritability estimates of larval growth of *Mytilus chilensis* under different food concentrations (Low, Medium, High). Nested design. Age (6, 12, and 20 days); number of individuals (N), average length in μm (Avg) with standard deviation (SD), heritability estimates for dam (h_d^2) and sire (h_s^2) components, and estimated mean change in growth (EMC = $i\,h_s^2$ SD) ($i = 10\%$ of selected animals) (Falconer, 1989).

Food level	Age (days)	N	Avg (μn)	SD (μn)	$h_d^2 \pm$SE	$h_s^2 \pm$SE	EMC (%)
Low	6	500	63	19	0.5±0.3	0.3±0.1	10.0
	12	550	86	22	0.4±0.3	0.2±0.1	7.7
	20	480	141	31	0.6±0.2	0.4±0.2	21.7
Medium	6	520	67	15	0.5±0.3	0.4±0.1	10.5
	12	600	97	18	0.7±0.2	0.4±0.2	12.6
	20	510	171	31	0.7±0.4	0.5±0.2	22.6
High	6	518	78	13	0.8±0.3	0.6±0.2	13.7
	12	506	122	21	0.7±0.1	0.6±0.1	22.1
	20	440	215	31	0.9±0.3	0.5±0.2	32.6

10% of the population for the trait, the estimated mean change in larval shell growth ranges between 7.7 and 32.6% per generation. Other studies (Mallet *et al.*, 1986; Stromgren and Nielsen, 1989) report the same conclusion for the blue mussel *M. edulis*. Shell length growth rate is closely correlated with meat weight increase in *M. edulis* juveniles (Nielsen, 1985), suggesting that selection for shell growth will at the same time increase the meat growth of mussels.

The result of our study imply that selection of *M. chilensis* growth characteristics could have practical implications in the commercial culture of this species.

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