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論 文 内 容 要 旨

Introduction

The scallops, bivalves of the family Pectinidae, have an increasingly economical value since they are mass produced for food. The seed obtainment is through collection from the sea, and also by artificial production under controlled conditions. The further culturing of the seeds can be done using suspended or releasing and bottom culture.

The genetic variability found in molluscs is generally high, and through natural and/or artificial selection some phenotypes are removed. The main problem is to drive the selection in the desired direction to obtain seeds of higher overall performance. To achieve this the genetics of these species must be studied.

1. Chromosome Number of the Chilean Scallop.

The purpose of this study was to determine the diploid chromosome number of the Chilean scallop species and compare it to the karyotypes obtained for the Japanese scallop, and other scallop species studied so far.

Early embryos (16 cells stage) obtained through artificial fertilization of the Chilean scallop, *Argopecten purpuratus*, were used and a modification of the Longwell & Stileas technique to remove the yolk was introduced. Squashing and staining were standard procedures.

The diploid chromosome number of the Chilean scallop is 2n=32, and of the Japanese scallop, *Patinopecten yessoensis*, is 2n=38, and the comparison of the chromosome morphology of both species is shown in Table 1.

Usually in molluscs the chromosome number is consistent within a family, like it occurs in Ostreidae (2n=20), Mytili-

dae (2n=38), and Veneridae (2n=38). In Pectinidae, instead, different chromosome numbers have been observed, like the species described before.

2. Electrophoretic Variability in Populations of the Chilean and the Japanese Scallop and the Genetic Diveregence between them.

The determination of the genetic similarities and differences is necessary for the possibility of using either one of these scallops as a model for the studies of scallop genetics.

The starch gel electrophoresis demonstrated that 19 isozymic loci are useful to analyze the Chilean scallop, and 10 out of them could be used to compare the Chilean and the Japanese species. The genetic variability found in both species was equally high, and no homozygote excess was observed neither in the hermaphroditic 1.5 year old Chilean, nor in the bisexual 4 year old Japanese scallop species. The calculated Nei's genetic distance between both species was 5.244, and all the results are summarized in Table 2.

3. Cold Shock Experiments at Early Stages of the Chilean Scallop.

Here, the relationship between a cold shock applied at an early stage of development of Chilean scallop larvae, and the growth and survival afterwards was studied, to find a non toxic, unexpensive selection agent.

Artificial spawning and fertilization of 4 animals (1 male and 3 females) was induced. 15 minutes after the fertilization 1 group was exposed during 15' to 0°C, and brought back to 22°C, the standard rearing temperature. The control group came from the same larval batch, mantaining all the conditions equal,

but rearing it continuously at 22°C.

The cold shock treated group showed a high mortality during the first growing period, but at the end of the hatchery phase, the survival of both groups was similar, as shown in Fig.1. The growth, under controlled conditions is shown in Fig.2 and was significantly higher in the treated group. The shell color composition of the cold shock group after 100 days did not show yellow or white morphs at all, present up to 10% in the control group. The early cold shock eliminates selectively the non resistant phenotypes, being these also linked to some shell color variants.

4. Shell Color Polymorphism and Growth in Japanese Scallop.

This study describes the existence of shell color polymorphism in the Japanese scallop, its abundance in different age classes, and a possible relationship between growth and/or survival with the described shell color polymorphism are discussed here.

The seeds of Japanese scallops for commercial cultures were obtained from the sea using collectors (Abashiri-Hokkaido). After 3 months the collectors are opened and a size selection is made, were the individuals over 9 mm are used for further culture, discarding the small sized scallops. The seed samples were taken at random, after opening the collectors and after selecting them, obtaining three different seed samples.

Three different shell color morphs could by distinguished according to the presence or absence of pigmentation on each valve, being therefore the morphs p/p, p/n and n/n. The p/n shells become predominant in the older age classes, and the n/n variant decreases during the scallop growth's, as shown in Table 3. The abundance of the non pigmented variants is reduced by size selection, being more frequent in the discarded seeds

than in the selected big seeds as it is shown in Table 4.

The not pigmented variant might be under the control of the recessive homozygote, and could be related with a lower survival as well as a tendency of lower growth.

5. Change of Homozygote Excess during the Development of Seed and Sown Populations of the Japanese Scallop.

The presence or absence of homozygote excess in different samples of seed and sown populations and the analysis of its presence in the different age and size classes, as well as the comparison of them, was the purpose of this study.

Four different samples of one year old seeds, as well as 2 and 3 year old samples of sown Japanese scallops were scored electrophoretically for a possible homozygote excess. 3 loci (*Aat-1*, α-*Gpd*, *Pgm*) showed a significant excess, and the overall excess was larger in the seed samples, decreasing with increasing age, becoming not significant at 3 years, and the results are summarized in Table 5. It was also possible to observe an higher homozygote excess in smaller individuals than in the larger belonging to the same sample.

A decrease of homozygosity during the development is considered to depend upon differentiated survival rates of homozygote and heterozygote individuals.

Final Conclusion

Using the different types of selection described, it is possible to eliminate early, at larva or seed stage, those phenotypes that are weak and would die during their development, or have a low overall performance, like slow growth and less resistance to environmental changes.

Table 1. Comparison of the Chromosome Morphology of the Chilean and the Japanese Scallop.

Chromosome Type	Chilean	Japanese
LM	4	0
M or SM	14	14
ST	6	18
T	8	6
	2n=32	2n=38

Table 2. Genetic Variability between the Chilean and the Japanese Scallop.

	Chilean Scallop	Japanese Scallop
N° of Loci used	10	10
Proportion of Polymorphic Loci	0.500	0.500
Average Heterozygosities		
observed (Ho)	0.186	0.177
expected (He)	0.187	0.181
Deviation from the Average Heterozygosity (Ho-He)/He	-0.005	-0.022

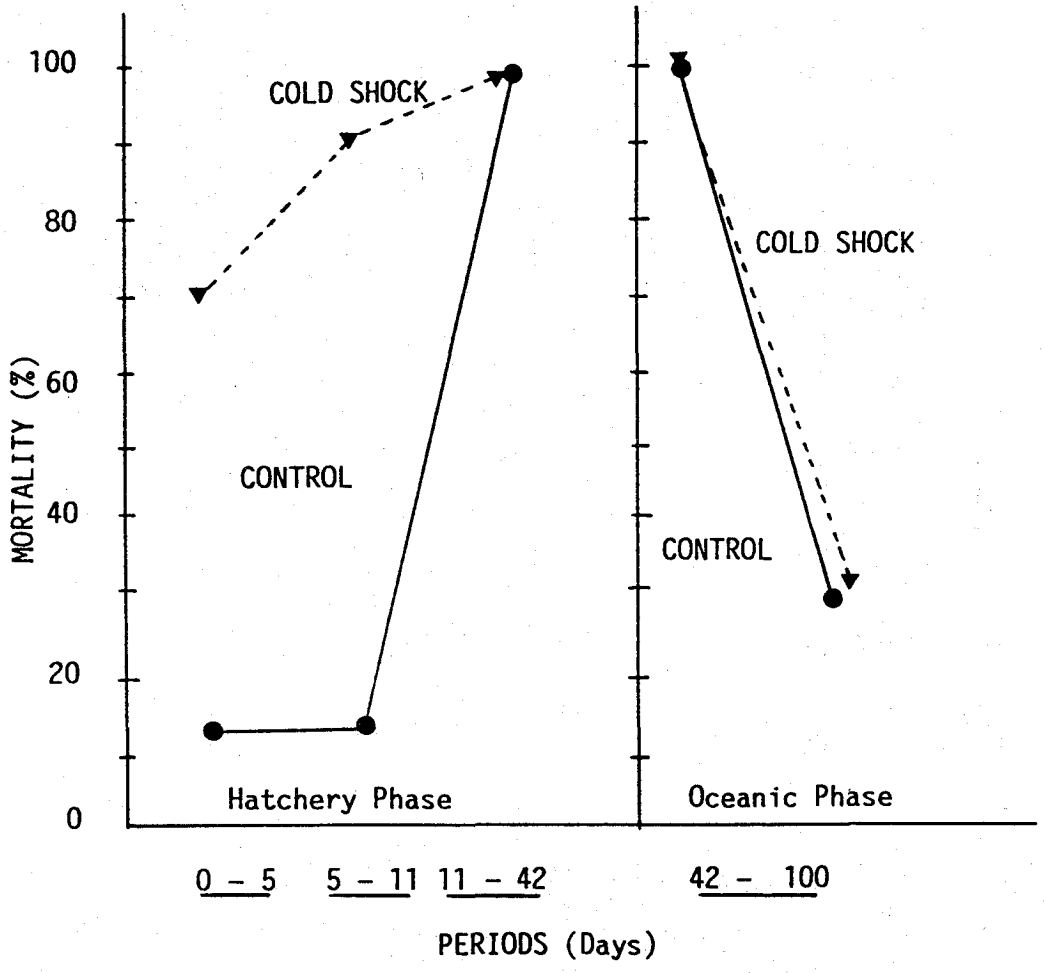

Fig. 1 Mortality (%) for Cold Shock treated and Control Group of Chilean Scallop.

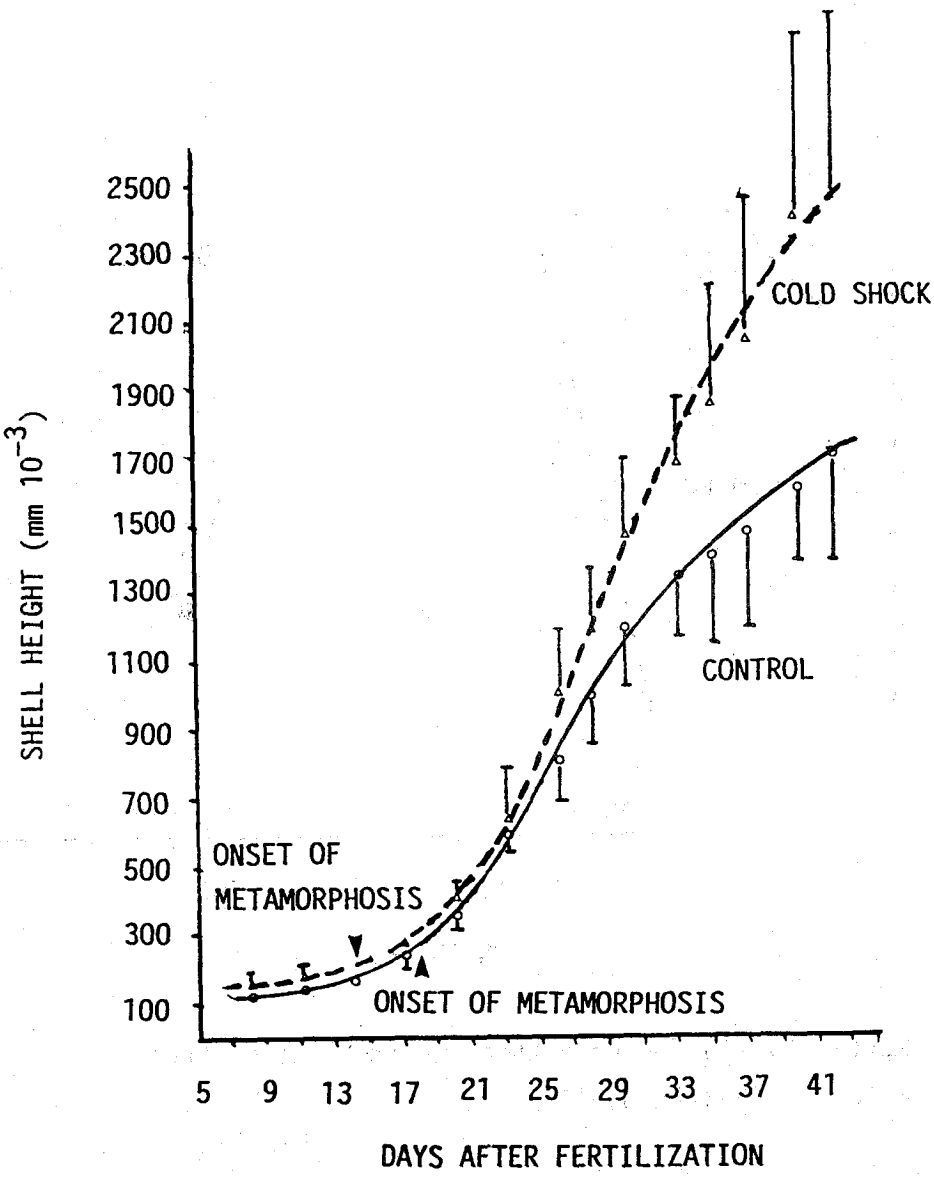

Fig. 2 Growth of Random (control) and Cold Shock Groups of chilean scallop A.purpuratus, in hatchery. (vertical bars represent 50% of sd)

Table 3. Shell Color Frequencies in different Age Classes and two Localities, of the Japanese Scallop P.yessoensis.

Locality	Age (year)	Color Morph (p/p)	(p/n)	(n/n)
Abashiri	0.25*	22	109	23
		(14.3%)	(70.8%)	(14.9%)
	1.00	31	65	4
		(31.0%)	(65.0%)	(4.0%)
	4.00	2	45	1
		(4.2%)	(93.7%)	(2.1%)
Notoro	0.25	26	120	17
		(16.0%)	(73.6%)	(10.4%)
	1.00	51	46	3
		(51.0%)	(46.0%)	(3.0%)

* corresponds to the random sample taken in this locality.

Table.4. Shell Color Frequencies of Different Sized Seeds of *Patinopecten yessoensis*.

Seed Type		Size	N	Color (p/p)	Color (p/n)	Color (n/n)
Random	R	11.4±2.7	154	22 (14.3%)	109 (70.8%)	23 (14.9%)
Small	S	8.6±1.1	100	14 (14%)	75 (75%)	11 (11%)
Big	B	12.1±1.6	235	43 (18.3%)	179 (76.2%)	13 (5.5%)

where N is the number of sampled individuals, and the number in parenthesis corresponds to the color frequency expressed in %.

The size corresponds to the mean shell height ± sd.

Table 5. Deviations of the expected heterozygosity at each locus in seed and sown populations of Japanese scallop

Locus		Seed population				Sown population	
		S1 (1)	S2 (1)	S3 (1)	S4 (1)	R1 (2)	R2 (3)
Aat-1	ho	0.322	0.307	0.333	0.361	0.226	0.323
	he	0.277	0.472	0.347	0.361	0.317	0.384
	χ^2	1.223	12.571*	0.075	0	4.757*	1.538
	(ho-he)/he	+0.162	-0.350	-0.040	0	-0.287	-0.159
Gpi	ho	0.177	0.043	0.204	0.198	0.137	0.160
	he	0.192	0.058	0.215	0.243	0.156	0.164
	χ^2	0.147	0.458	0.073	1.094	0.325	0.012
	(ho-he)/he	-0.078	-0.259	-0.051	-0.185	-0.122	-0.287
αGpd	ho	0.351	0.527	0.495	0.474	0.525	0.515
	he	0.590	0.637	0.643	0.588	0.582	0.548
	χ^2	26.818*	5.839*	9.514*	5.164*	1.584	0.444
	(ho-he)/he	-0.405	-0.173	-0.230	-0.194	-0.098	-0.060
Mdh-1	ho	0	0.009	0	0.031	0	0.020
	he	0	0.008	0	0.030	0	0.020
	χ^2	0	0.011	0	0.004	0	0
	(ho-he)/he	0	+0.125	0	+0.033	0	0
Pgm	ho	0.470	0.470	0.545	0.417	0.410	0.567
	he	0.502	0.626	0.661	0.579	0.473	0.592
	χ^2	0.476	11.890*	5.855*	10.136*	2.000	0.246
	(ho-he)/he	-0.064	-0.249	-0.175	-0.280	-0.133	-0.042
6Pgd	ho	0	0.026	0.071	0.082	0.032	0.030
	he	0	0.043	0.105	0.079	0.077	0.030
	χ^2	0	0.770	1.180	0.023	3.448	0
	(ho-he)/he	0	-0.395	-0.324	+0.038	-0.584	0
Overall	Ho	0.220	0.230	0.275	0.260	0.222	0.269
	He	0.260	0.307	0.329	0.313	0.267	0.290
	(Ho-He)/He	-0.154	-0.251	-0.164	-0.169	-0.169	-0.022

The number in parentheses represents the age class.

*: not agreeing with the Hardy-Weinberg's equilibrium.

-: excess of homozygosity.

+: deficiency of homozygosity.

審査結果の要旨

ホタテガイの養殖は，天然において発生した幼生を付着期に採苗し，海面で飼育した後に大きさによって選択し，中間育成を経て地蒔放流，または垂下養殖によって生産されている。ホタテガイは大量の幼生を産出するが，そのうち成貝まで生残するのは少数であり，この過程は種々の自然および人為選択を受けていると考えられる。一方，海産二枚貝類は魚類に比べて顕著に高い遺伝的変異性を持ち，集団中に多くの有害遺伝子を保有していることが予想されていることから，これらの選択がどのような遺伝的組成の個体に対して行われているかを明らかにできれば，有効なホタテガイの養殖種苗生産方法を育種学的に検討できる。

本研究はこの観点に立ち，雌雄同体のチリ産ホタテガイと日本産ホタテガイの種苗及び成貝の遺伝的特徴を明らかにし，選択がホタテガイの養殖集団の遺伝的組成に及ぼす効果を解析している。

第一にチリのホタテガイの染色体数を調べ，２ｎ＝32であることを明らかにした。一般に二枚貝類は科によって一定の染色体数を示すが，これは日本のホタテガイの２ｎ＝38より少なく，ロバートソン型融合によると推定している。次に，両種のアイソザイム遺伝子による遺伝的分化の程度と遺伝的変異性を調べ，両種の分化程度は大きいが，雌雄同体であるチリのホタテガイが雌雄異体である日本のホタテガイと同レベルの高い遺伝的変異性を示すことを明らかにし，チリのホタテガイも異系交配していることを示した。

ホタテガイの初期発生での温度による選択の効果を明らかにするために，チリのホタテガイをもちいて受精卵の低温処理と無処理の死亡率と成長を比較した結果から，初期選択の有効性を示した。また，この時の生残個体の殻色は淡色の個体が無くなっていることから，殻色と生存性の関係を示唆した。

そこで日本のホタテガイの殻色の分布を中間育成前，種苗放流時，成貝で比較したところ，年齢が進むに連れて無殻色の個体の割合が少なくなることを明らかにした。これにより劣性ホモ個体が選択されている可能性を示唆した。

そこでアイソザイム遺伝子を用いて放流集団と放流後の集団の遺伝的組成の差異を調べ，放流集団にみられたホモ接合体過剰が放流後に認められなくなっていることを示し，ホモ接合体が選択されていることを明らかにした。これらのことからホタテガイの種苗生産には初期に有害遺伝子を除去するような選択が効果的であることを示した。よって，農学博士の学位を授与するに充分な価値があると判断した。