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By

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The prevailing classification of the oyster based on the conchological characters is by no means a concrete one, since the shell character varies readily with changes of the environmental conditions. Even now, there are some confusions among taxonomists in identifying the species. Tomita and Koizumi (12), using a serological method, were able to differentiate antigens among four genera of bivalves and between *Ostrea gigas* and *Ostrea circumpicta.** In the present study, ring test and absorption test were made with an attempt to find the possibility of using the serological analysis for identifying the closely related species and races of oysters and also for the estimation of affinities among species.

Materials Used

In the present investigation, the following four species and four local races of oyster were used:

1) *Ostrea edulis* (European flat oyster)
2) Four local races of *Crassostrea gigas* (Japanese oyster), namely;
   Kumamoto race: Syn. *Ostrea gigas* var. *sikamea* Amemiya
   Hirosima race: Syn. *Ostrea laperousei* Schrenck
   Miyagi race: Syn. *Ostrea gigas* Thumberg
   Hokkaido race: Syn. *Ostrea gigas* Thumberg
3) *Crassostrea angulata* (Portuguese oyster)
4) *Crassostrea rivularis*

Ostrea edulis is a larviparous oyster, while the other three species which belong to the genus *Crassostrea* are oviparous. *Crassostrea gigas* has been

* According to the present classification, *Ostrea gigas* and *Ostrea circumpicta* should be revised as *Crassostrea gigas* and *Crassostrea nippona* respectively.

given various names by different authors such as Thumberg (11), Schrenck (9), and also has been discussed rather frequently from taxonomic viewpoint by Hirase (3), Wakiya (13), Amemiya (1), Takatsuki (10), Gunter (2), Ranson (8). Recently Imai *et al.* (4, 5, 6, 7,) studied the local forms of *C. gigas* of around Japan systematically by breeding experiments and concluded that *O. laperousei*, *O. gigas* and *O. gigas* var. *sikamea* belong to a single species, *Crassostrea gigas*, and they were able to distinguish four local races with different characters in *Crassostrea gigas* of Japan.

The portuguese oyster, *Crassostrea angulata* and the Japanese oyster, *Crassostrea gigas*, resemble each other very closely and it is difficult to distinguish one from the other by mere inspection. As a result of researches on the interspecific cross-breeding between the two species, Imai *et al.* (7) found that, fertilization, larval development and setting proceeded normally and they were able to raise the hybrids to mature. However, so far as their researches went, the larvae of F_2 generation ceased to develop over 150μ in height. Therefore, they were not able to raise F_2 generation to the adult form.

Both *Crassostrea rivularis* and the Kumamoto race of *C. gigas* are found in the same localities. As a result of breeding experiment, it was confirmed that *C. rivularis* is a distinct species and differs from both *C. gigas* and *C. angulata*.

Experimental Method

Preparation of antigens. The antigens used in the experiments was prepared from the bloods of oysters mentioned above. The blood was obtained directly from the auricle by means of a syringe, and them it was centrifuged to separate the serum.

Preparation of antisera. Rabbit antisera against the serum of each species of oysters, excluding the Miyagi and Hokkaido races of *C. gigas*, were prepared by intervenous injections. To the serum and antiserum thus obtained, methiolate was added giving a final concentration of 1 : 10,000, in order to prevent the bacterial growth and they were stored for subsequent test. The addition of preservative did not affect the serological reactions.

Ring test. Both antigen and antiserum were diluted, and tests were made between them in various combinations of dilutions. The dilution was made in 10×2^n for antigen and in 2^n for antiserum, by using sterile physiological saline water buffered to a pH of 7.0. Readings were made at the end of one hour at 37°C, then test tubes were kept at room temperature for two or three hours until final readings were made. From the results of the series of ring tests, diagrams of zone of the reaction as shown in Fig. 1. was obtained.

Absorption test. Absorption test was tried to make clear the relationship

among the species and races. The centrifuged supernatants of the tubes which showed negative reactions to further addition of antigen were used as absorbed serum in the subsequent ring test. Since the serum of the oyster used as antigen in the test contained several antigenic substances in mixture, the ratio of antigen titer: antiserum titer of the zone of reaction in ring test can not be used directly as an index of optimal proportion in the absorption test. In fact, the amount of antigen used for absorption far exceeded the amount calculated from the ratio of antigen titer: antiserum titer in the ring test.

Results and Discussion

Homologous ring test. The results of the homologous ring test in four species and two races are shown in Fig. 1. and Table 1. It is apparent that two dimensional diagrams obtained for three species within the same genus, *C. gigas*, *C. angulata* and *C. rivularis*, are similar to each other in shape, while that of *O. edulis* is quite different from the others.

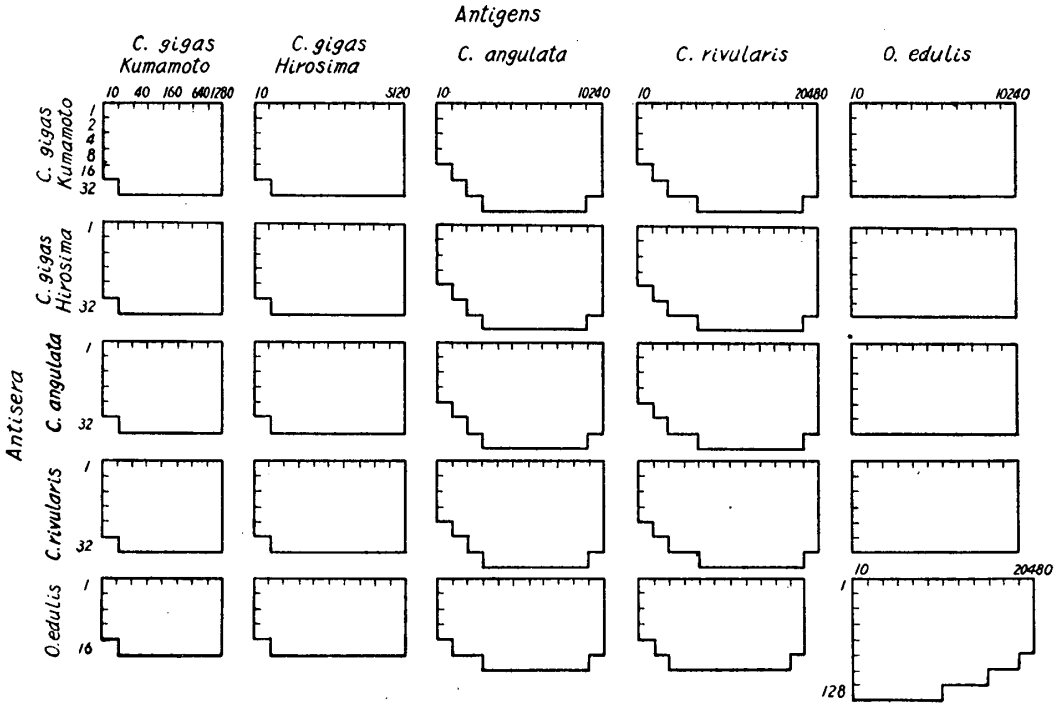

Fig. 1. Diagrams of zones of the homologous and heterologous precipitin reactions in four species and two local races of oysters. The numbers shown on the upper side of each diagram represent dilutions of antigen, and those on the left side, dilutions of antiserm.

Heterologous ring tests. Heterologous ring tests were made in all the combinations of the four species and two races, excluding the Miyagi and Hokkaido races of *C. gigas*. The two dimensional diagrams and both titers

are shown in Fig. 1. and Table 1. The titers and the shape of diagrams between the three species within *Crassostrea* were the same, when each antisera were tested to the same antigens respectively. And when the antisera to these three species were tested with the *O. edulis* antigen, antigen and antiserum titers were of the same order of magnitude but both titers of these heterologous tests were markedly lower than those of homologous test of *O. edulis*. And, when antiserum to *O. edulis* were tested with antigens of these three species, the antiserum titers were lower than in those of respective homologous tests.

Table 1. Antigen and antiserum titers of both homologous and heterologous reactions in four species of oysters.

Antisera	Antigens				
	C. gigas Kumamoto race	*C. gigas* Hirosima race	*C. angulata*	*C. rivularis*	*O. edulis*
C. gigas Kumamoto race	1280 (32)	5120 (32)	10240 (64)	20480 (64)	10240 (32)
C. gigas Hirosima race	1280 (32)	5120 (32)	10240 (64)	20480 (64)	10240 (32)
C. angulata	1280 (32)	5120 (32)	10240 (64)	20480 (64)	10240 (32)
C. rivularis	1280 (32)	5120 (32)	10240 (64)	20480 (64)	10240 (32)
O. edulis	1280 (16)	5120 (16)	10240 (32)	10240 (32)	20480 (128)

Note ; Each figure represents the antigen titer, and the figure in parentheses the antiserum titer.

At the present stage of study, no conclusive explanation can be given regarding the differences in shape of the two-dimensional diagrams, but it can be said that by ring test we can differentiate antigens between different genera.

Absorption test. Because of the occurrence of common or similar antigens in the serum of four species, it is difficult to differentiate antigens between species and between races unless the antisera is absorbed with the antigens of the other species in advance. Therefore, the absorption tests were tried between four species including four races of *C. gigas*, to find the relationships among species and races. The results are summarized in Table 2.

Antiserum to the Kumamoto race of *C. gigas* was absorbed with antigens of *C. angulata*, *C. rivularis* and *O. edulis* respectively. Each absorbed antiserum thus prepared showed no reactions with antigens of *C. angulata*, *C. rivularis* and *O. edulis*, but reacted strongly with antigens of the four races of *C. gigas*. The same results were obtained in antiserum to the Hirosima race of *C. gigas*. And both antisera to the Kumamoto and to Hirosima races of *C. gigas*

absorbed with antigens of each races of *C. gigas* have no antibodies against other species but against certain races of *C. gigas*. From the results mentioned above, it can be said that four races of *C. gigas* have gigas-specific

Table 2. Summary of absorption tests obtained. +indicates positive reactions, −indicates negative reactions.

Antisera	Antigens added to absorb	Antigens						
		G.K.	G.Hi.	G.M.	G.Ho.	A.	R.	E.
G.K.	G.Hi.	+	−	−	−	−	−	−
	G.M.	+	−	−	−	−	−	−
	G.Ho.	+	+	−	−	−	−	−
	A.	+	+	+	+	−	−	−
	R.	+	+	+	+	−	−	−
	E.	+	+	+	+	−	−	−
G.Hi.	G.K.	−	−	−	−	−	−	−
	G.M.	+	+	−	−	−	−	−
	G.Ho.	+	+	−	−	−	−	−
	A.	+	+	+	+	−	−	−
	R.	+	+	+	+	−	−	−
	E.	+	+	+	+	−	−	−
A.	G.K.	−	−	−	−	+	−	−
	G.Hi.	−	−	−	−	+	−	−
	G.M.	−	−	−	−	+	−	−
	G.Ho.	−	−	−	−	+	−	−
	R.	−	−	−	−	+	−	−
	E.	−	−	−	−	+	−	−
R.	G.K.	−	−	−	−	−	−	−
	G.Hi.	−	−	−	−	−	−	−
	G.M.	−	−	−	−	−	−	−
	G.Ho.	−	−	−	−	−	−	−
	A.	−	−	−	−	−	−	−
	E.	−	−	−	−	−	−	−
E.	G.K.	−	−	−	−	−	−	+
	G.Hi.	−	−	−	−	−	−	+
	A.	−	−	−	−	−	−	+
	R.	−	−	−	−	−	−	+

Note; G.K.=*Crassostrea gigas* Kumamoto race, G.Hi=*Crassostrea gigas* Hirosima race
G.M.=*Crassostrea gigas* Miyagi race, G.Ho.=*Crassostrea gigas* Hokkaido race
A. =*Crasscstrea angulata*, R.=*Crassostrea rivularis*, E.=*Ostrea edulis*

antigen in common respectively which is absent in the other species, and Imai *et al.* were correct in classifying the oyster populations of four localities as local races in *Crassostrea gigas* and not as different species. The antisera to *C. angulata* absorbed with antigens of *C. gigas*, *C. rivularis* and *O. edulis* respectively reacted with antigen of *C. angulata*, but not with that of other species. And also, the antisera to *O. edulis* absorbed with antigens of other species reacted with antigen of *O. edulis* but not with other three species. But, the antisera to *C. rivularis* absorbed with antigens of other species did not react with antigens of other species and even not with that of *C. rivularis*.

As regards to the species relationships, a simple explanation of the result

will be that these four species have common or a similar antigen in common and that three of them have also the species specific antigen respectively but not in *C. rivularis*.

As regards to the relationships among four local races of *C. gigas*, it can be seen that four races have species specific antigen in common, that Kumamoto has another antigen in addition which is not found in the other three races and that the Hirosima race also has another antigen which is not found either in the Miyagi and the Hokkaido races. From the fact that antiserum to the Kumamoto race absorbed with antigen of the Miyagi race showed no reaction with antigen of the Hirosima race but antiserum to the Hirosima race absorbed with antigen of the Miyagi race reacted with antigen of the Hirosima, it can be assumed that the Hirosima race has antigen which is not found in the Kumamoto race, though antigen of the Kumamoto race removed all antibodies from antiserum to the Hirosima race. It can be presumed that an antigen in the Kumamoto race has a structure similar to the antigen specific to the Hirosima race and that the reaction between the antiserum to the Kumamoto race absorbed with antigen of the Miyagi race and antigen of the Hirosima race is inhibited by simple hapten of the Miyagi race. There remains much to be solved regarding races, since antisera to the Miyagi and Hokkaido races were not prepared. In the present project, we are continuing the studies by means of the precipitin technique with higher sensitivity in order to determine the antigenic properties of oyster species and races in more detail.

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Summary

1) The serum of four species and two local races of the oyster were tested to determine the zone of precipitin reaction with the corresponding rabbit antisera in all the combination of dilution.

2) Consistent differences in the zone of precipitin reaction were found between *Ostrea edulis* and three species of *Crassostrea*. But the difference was hardly recognized among species of the genus *Crassostrea*.

3) In absorption test, it was found that four species have common or similar antigen respectively and three of them have their own species specific antigen in addition but *C. rivularies* has not.

4) A few informations regarding the relationships of four local races of

C. gigas were also obtained. Absorption technique may be useful in detecting antigenic differences among oysters at race levels.

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