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Title	Studies on the Systematic Serology in Sea-Stars, I (With 3 Text-figures)
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Citation	北海道大學理學部紀要, 13(1-4), 67-71
Issue Date	1957-08
Doc URL	http://hdl.handle.net/2115/27203
Type	bulletin (article)
File Information	13(1_4)_P67-71.pdf

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Studies on the Systematic Serology in Sea-Stars, I

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(*With 3 Text-figures*)

Although still greatly dependent upon traditional morphological method, taxonomic research has yet come to be substantially supplemented and expanded by serology, due primarily to the impetus given this type of research method, in the field of animal systematics, by Nuttall (1904). But with the exception of a recent attack by Wilhelmi (1942) on the problem of the serological distinctions and relationships among the three classes of Echinoderms, little work has been done, as far as the writer is able to determine, concerning a systematic serology of sea-stars. In view of this situation, the present studies were undertaken.

The writer wishes to express his sincere thanks to Professor Tohru Uchida of Hokkaido University for kind guidance, and to Professor R. Hayashi who made many helpful suggestions and identified the species examined. Thanks are due also to Dr. M. Sugiyama, Director of the Sugashima Marine Biological Station of Nagoya University, for his kindness in providing facilities for collection of materials, and particularly to Dr. Y. Watanabe for active interest, criticisms and assistance in preparation of the manuscript.

Material and method

The following four species, obtained from the coast of Sugashima, Mie Prefecture, were the materials used for the present investigation : *Coscinasterias acutispina* (Stimpson), *Asterina pectinifera* (Müller et Troschel), *Astropecten scoparius* Valenciennes and *Certonardoa semiregularis* (Müller et Troschel).

For the preparation of antigenic solution, the ambulacral zone, consisting chiefly of tube feet and skeleton, was snipped off from the animal body, well minced with a pair of scissors and ground in a mortar to be extracted by a 1/2 M NaCl solution at pH 7.4 with phosphate buffer. The extract was dialized against distilled water, and to avoid superheating of the extract solution above 25°C, was carefully condensed with a vacuum distillater, after which it was lyophilized. Antigen employed, for both antibody-production and serological reactant, was prepared by re-dissolution in a 0.85 per cent NaCl solution buffered at pH 7.4, with "Merzonin" (Takeda preparation) at a concentration of 1 : 10,000 being added as a preservative.

The rabbits were intravenously injected every two days with successively increasing dosages of antigen, *viz.*, 15, 45, 75 and 115 mg in dry weight, *i.e.*, 250 mg in total ; 7 to 10 days after the last injection they were exsanguinated from the carotid. The antiserum thus produced was expressed from the blood clot,

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 13, 1957 (Prof. T. Uchida Jubilee Volume).

mixed with merzonin (1 : 10,000 in final concentration), and stored in a refrigerator until use. In the manner described above, anti-*Asterina pectinifera*, anti-*Certonardoa semiregularis* and anti-*Coscinasterias acutispina* sera were prepared.[1)]

The examination of the precipitin reaction system was made turbidimetrically with a photoelectric colorimeter, the principle and technique here being essentially the same, but with slight modifications, as those of the Heidelberger method (cf. Kabat and Mayer, 1948). For an economincal use of the antisera prepared, prior to the test, they were diluted to 1 : 2 or 1 : 3 with a 0.85 per cent NaCl solution. Increasing amounts of antigen were added to a constant volume (0.5 ml) of antiserum in a series of vials, mixed thoroughly and incubated 1 hour at 37°C, and then allowed to stand 2 days in a refrigerator at a temperature of about 0°C. After the mixture were centrifuged at room temperature (9–12°C), the precipitates were separated, washed three times with a cold and buffered 0.85 per cent NaCl solution, and dissolved in 0.6 ml of a 1/10 M NaOH solution. The dissolved reaction-products were again precipitated with 0.1 ml of a 20 per cent sulfosalicylic acid, and then mechanically shaken 30 minutes to disperse into a homogeneous fine suspension in the liquid with a glass stirrer being introduced as an aid. Light transmittance of the suspension was immediately measured with the colorimeter, and its turbidity was computated as $\log_{10}$ of the reciprocal of the photometric intensity reading on the galvanometer, which computation was used as an index for anitgen-antibody reaction. The average turbidities obtained from duplicate tests of a whole series of antigen amounts were plotted as against successive amounts of antigen in mg. To compare heterologous antigens with homologous standard of reference, the difference or variation in titration curve thus made was examined.

For the comparison of a variety of antigens, as was pointed out by Boyden and DeFalco (1943), it is not satisfactory to measure amounts of settled precipitate only at a particular amount or amounts of antigens, but it is also necessary to examine the reaction of antiserum in accord with a sufficiently wide range of antigen amount. In fact, even in the present work, with maximal deviation from the average being found to be ±0.0049, ±0.0077 and ±0.0148 in the turbidity scale unit for, respectively, the anti-*Certonardoa*, the anti-*Asterina* and the anti-*Coscinasterias* serum, no demonstrable difference could be detected among the different reaction systems when tested only with antigens of a particular amount. The present method, however, offers no difficulty in determining serological similarities or differences among these sea-star extracts.

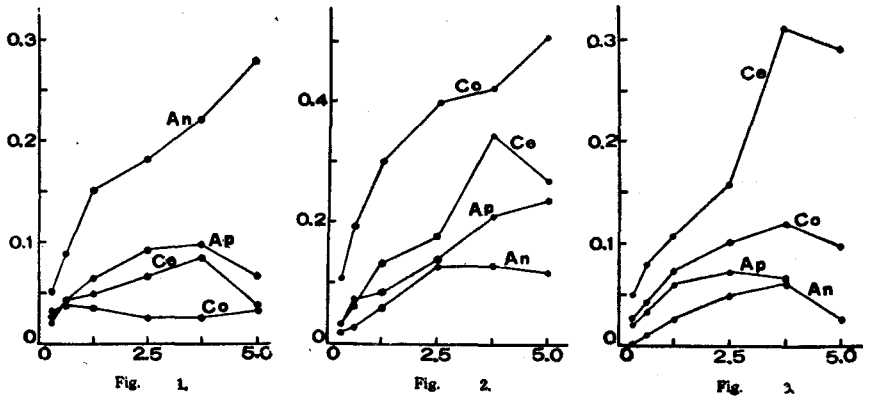
Results

The experimental results are summarized in graphs. The data from the

1) For convenience, only generic names are used in the following pages, *e.g.*, anti-*Asterina* serum, *etc.*

anti-*Asterina* serum are given in Figure 1, those from the anti-*Coscinasterias* serum in Figure 2, and those from the anti-*Certonardoa* serum in Figure 3. These graphed data all agree in showing, first, that the homologous antigen does, in general, greatly differ from any of the heterologous antigens as regards shape and height of the titration curve, or serological correspondence : and second, that the titration curve or serological correspondence, of heterologous reaction differes in different combinations of antigen and antiserum. With the anti-*Asterina* serum, as shown in Figure 1, the serological correspondence of the *Asterina* (homologous) antigen is highest; next comes the *Astropecten* antigen; the serological correspondence of the *Certonardoa* antigen is low; and that of the *Coscinasterias* antigen is still lower. That is to say, for the anti-*Asterina* serum serological correspondences among these four antigen species give a graded series :

Asterina>*Astropecten*>*Certonardoa*>*Coscinasterias*



Figs. 1–3. Titration curves of the anti-*Asterina* (Fig. 1), the anti-*Coscinasterias* (Fig. 2) and the anti-*Certonardoa* serum (Fig. 3) with the antigens from *Asterina* (An), *Astropecten* (Ap), *Certonardoa* (Ce) and *Coscinasterias* (Co). Ordinates : turbidity as defined in the text (p. 70); abscissae : antigen amount in mg. The anti-*Asterina* and anti-*Coscinasterias* sera are diluted to 1 : 2, and the anti-*Certonardoa* serum to 1 : 3 with a buffered saline solution, respectively.

Similarly, with each of the anti-*Coscinasterias* and anti-*Certonardoa* sera (Figs. 2 and 3), the antigens tested can be arranged according to their difference from the corresponding homologous standard locus as follows :

For the anti-*Coscinasterias* serum ;

Coscinasterias>*Certonardoa*>*Astropecten*>*Asterina*

For the anti-*Certonardoa* serum :

Certonardoa>*Coscinasterias*>*Astropecten*>*Asterina*

The serological correspondence is consitently lowest in a combination of either *Asterina* antigen with anti-*Coscinasterias* serum or *Coscinasterias* antigen with anti-*Asterina* serum. Moreover, in these four antigen species, the ranking order of reactivity with the anti-*Coscinasterias* serum is just the reverse of that shown with anti-*Asterina* serum, thus suggesting that *Asterina* and *Coscinasterias* are serologically more distant from each other than they are from either *Astropecten* or *Certonardoa*. The antigen order obtained for the anti-*Certonardoa* serum indicates that *Certonardoa* is more closely related to *Coscinasterias* than to *Astropecten*, and bears very little relation to *Asterina*. The results from the experiments with the anti-*Asterina* and the anti-*Coscinasterias* serum do not exclude the possibility of these interrelations in *Certonardoa*, *Coscinasterias* and *Asterina*. *Astropecten* appears in general to be nearer the locus of *Asterina* than it is to that of either *Certonardoa* or *Coscinasterias*, though the serological relations of *Astropecten* to the other three remain to be conclusively determined, since in the present investigation, a reciprocal test was not made with the anti-*Astropecten* serum, for lack of sufficient preparetion of it.

General considerations

According to Fisher's extensive monograph on the Asteroidea (1911). *Asterina* belongs to the order Spinulosa, and *Coscinasterias* to the Forcipulata, while both *Astropecten* and *Certonardoa* are included in another order, Phanerozonia. As evidenced by the preceding data, the serological relation of *Asterina* to *Coscinasterias* is unquestionably distant. This may favor the view of Fisher (1911) that the Spinulosa is sharply separable from the fairly well defined order, Forcipulata. Between *Astropecten* and *Certonardoa*, however, the serological relation seems not to be so close, as they have been placed in the same collective category. Even in morphological aspect, these two genera are so notably different from each other that they have been assigned to two different suborders of the Phanerozonia, i.e., *Astropecten* to the Paxillosa, and *Certonardoa* to the Valvata (Fisher, 1911 and 1930).

The taxonomic position of the family Linckiidae, including *Certonardoa* is widely varied in the systems thus far presented by the sea-star specialists. Sladen (1889) divided the Asteroidea into two orders, the Cryptozonia and Phanerozonia, and regarded the Linckiidae as belonging to the former. In contrast, Fisher (1911) rearranged Verrill's revision of Sladen's classification, and transferred the Linckiidae to his Phanerozonia, but he assigned most of Sladen's cryptozoniate forms, among which was the present *Coscinasterias acutispina* (=*Asterias acutispina*), to the Forcipulata. Fisher stated, however, that, "in determining the order Phanerozonia, the ambital skeleton and distribution of the respiratory papulae are used, although in the tansitional or intermediate family Linckiidae these characters are variable which is tantamount to saying that the order is not sharply defined" (Fisher, 1911, pp. 18–19). By reference to the above

mentioned serological relations among *Certonardoa*, *Coscinasterias* and *Astropecten*, both the statement and classification of Fisher, as well as Sladen's arrangement, are of deep interest, in so far as they suggest that the Linckiidae is an aberrant group of the Phanerozonia, retaining, however, a relatively intimate connection with the Forcipulata, rather than otherwise. Contrary to the Linckiidae, the family, Asterinidae, to which *Asterina pectinifera* belongs, have been removed by Fisher from Sladen's Phanerozonia to the Spinulosa, while the Astropectinidae is assigned to his Phanerozonia. But as he repeatedly remarked, the order Spinulosa is not adequately defined as regards its limits toward the Phanerozonia (Fisher, 1911). In consideration of such an unstable situation in taxonomy, it appears not altogether strange that in serology, *Asterina* probably lies near the locus of a phanerozoniate form, *Astropecten*. On the morphological side, however, there are no comparable data yet available to prove the relation between these two forms to be convincingly as such.

As an outcome of these considerations, the writer is inclined to the view that the order Phanerozonia appears to be a heterogeneous assemblage of a variety of sea-star groups, probably some of which have an affinity to the Forcipulata, others to the Spinulosa and still others have neither such relation, though these two orders apparently stand far apart from each other. This remains hypothetical, however, and further investigation is unquestionably necessary.

Summary

To establish the serological relations among the four sea-star species, *Coscinasterias acutispina*, *Asterina pectinifera*, *Astropecten scoparius* and *Certonardoa semiregularis*, the precipitin reaction systems were examined turbidimetrically with a photoelectric colorimeter. The serological correspondences obtained from various combinations of antigen and antiserum revealed: 1) *Asterina* and *Coscinasterias* are serologically more distant from each other than they are from either *Astropecten* or *Certonardoa*; 2) *Certonardoa* is more closely related to *Coscinasterias* than to *Astropecten*, and bears very little relation to *Asterina*; 3) *Astropecten* appears to be nearer the locus of *Asterina* than it is to that of either *Certonardoa* or *Coscinasterias*. A brief discussion on the taxonomic classification is appended.

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