Failure of interbreeding between Atlantic and Pacific populations of the marine calanoid copepod *Acartia clausi* Giesbrecht[1]

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Abstract

An attempt has been made to interbreed populations of *Acartia clausi* from Woods Hole, Massachusetts, and Yaquina Bay, Oregon. Individuals from these populations are morphologically similar, but differ somewhat in size. Cultures from both sources produced many successive generations in the laboratory, but interbreeding with production of viable offspring did not occur. This is evidence that the Atlantic and Pacific populations have diverged in isolation to the level required to be assigned specific rank.

The nomenclature of organisms is primarily based on morphological differences as the decisive criteria for assignment to specific rank. The degree to which species described on morphological grounds fall within the biological species definition (Mayr 1963) is always difficult to evaluate. Assessment of reproductive isolation by interbreeding experiments with marine organisms has been greatly hampered by the difficulty of culturing these animals. For this reason, little work has been done on interbreeding allopatric populations of conspecific copepods, though they are the most abundant group of animals in the marine environment. An exception is the work done with allopatric populations of nearshore harpacticoid copepods: reduced fertility was found in some crosses between *Tisbe reticulata* Bocquet from Britanny and from the Venetian lagoon (Battaglia 1956) and between various populations of *Tigriopus fulvus* (Bŏzić 1960).

This paper reports some interbreeding experiments between allopatric, conspecific populations of the marine calanoid copepod *Acartia clausi* Giesbrecht. East and west coast populations of *A. clausi* were chosen as experimental organisms because of their broad distribution, and because the difficulties associated with culture of *Acartia* for several generations under laboratory conditions have been overcome by Zillioux (1969), Heinle (1969), and others.

Giesbrecht (1889) briefly described *A. clausi* from specimens from the Mediterranean. He later (Giesbrecht 1892) noted its occurrence in the eastern North Atlantic Ocean and North Sea. This was confirmed by Steuer (1923) and others. The existence of populations in the western North Atlantic was described by Herdman et al. (1898) and Williams (1906), and confirmed by Fish (1925), Bigelow (1926), and many others. Populations in the eastern North Pacific have been referred to as *A. clausi* by several usually reliable copepod taxonomists (Esterly 1924; Fleminger 1964), and this judgment has been generally accepted by other investigators. Areas of occurrence of *A. clausi* are found in all north temperate seas (Steuer 1933). According to Grainger (1965) the distribution of *A. clausi* is apparently disjunct through the Arctic Ocean. The distribution of *Acartia longiremis* (Lilljeborg) is more nearly continuous through the Arctic, although its full continuity remains to be established (Grainger 1965). A breeding experiment between Atlantic and Pacific populations of that species

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Table 1. Summary of interbreeding attempts between Atlantic and Pacific populations of Acartia clausi. *A—Atlantic; P—Pacific.*

Date started	Source and no. of adults at beginning		No. alive after 1 week		No. of nauplii alive at end	No. of ♀ carrying a spermat. at end
	♀	♂	♀	♂		
29 May*	5A	5P	1	5	2	1
28 Jun	3A	4P	3	4	0	0
28 Jun	3A	4P	3	3	0	0
2 Jul†	5A	4P	3	1	0	0
4 Jul	3A	4P	2	3	0	0
5 Jul	3A	3P	2	0	0	0
11 Jul†	4A	3P	2	1	0	0
17 Jul	6A	4P	5	4	0	1
17 Jul†	6A	5P	6	4	0	0
17 Jul†	6A	1P	6	1	0	0
17 Jul†	2A	2P	2	1	2	0
14 Jun	6P	6A	5	4	0	1
14 Jun	6P	6A	5	4	0	0
17 Jul	5P	1A	2	1	0	0
23 Jul	4P	3A	4	2	0	0
23 Jul	4P	3A	4	2	0	0

* Atlantic females obtained from 9 March 1973 culture, not individually reared.

† Males not individually reared.

Table 2. Summary of control beakers for Atlantic and Pacific populations of Acartia clausi.

Source and No. of Adults at beginning		Date ended	No. alive after 1 week		No. of nauplii alive at end
♀	♂		♀	♂	
3	4 AT	4 Jul	3	2	71
3	4 AT	4 Jul	1	1	88
6	1 AT	9 Jul	6	1	45
3	2 AT	11 Jul	2	0	18
3	3 AT	14 Jul	3	1	107
6	4 AT	16 Jul	1	3	> 25
7	3 AT	22 Jul	5	2	> 25
7	3 AT	23 Jul	4	3	> 25
6	3 AT	23 Jul	2	1	> 25
4	3 AT	30 Jul	3	2	55
4	3 AT	30 Jul	2	2	33
3	4 PAC	4 Jul	1	4	12
3	4 PAC	4 Jul	2	4	64
6	6 PAC	21 Jun	5	6	80
6	6 PAC	21 Jun	4	4	67
7	5 PAC	10 Jul	3	4	29
3	8 PAC	12 Jul	1	3	14
4	3 PAC	30 Jul	3	3	89
4	3 PAC	30 Jul	4	2	48

would have made an interesting comparison, but we have not yet been successful at culturing it.

Acartia clausi belongs to the large section of calanoid copepods (Heterarthrandria) in which the male actively feeds and can survive a substantial portion of the maximum life span of the female. The female can be fecundated at any time after molting to the adult stage. These characteristics make this species preferable for this work to any of the common amphascandrid copepods such as *Pseudocalanus* in which the females must be fecundated at the time of the adult molt, and the male is nonfeeding and short lived.

The idea for this experiment came from S. Zimmerman. We thank A. Carey, B. Frost, and A. G. Lewis for critical comments.

Methods and materials

All the copepods used in this work were collected at Yaquina Bay, Oregon, or at Woods Hole, Massachusetts. They were captured with ring nets equipped with mesh of 0.239 mm or finer. Pacific collections were made on five occasions from June 1971 to February 1973; Atlantic collections were made on four occasions from September 1972 to June 1973.

Upon capture at Yaquina Bay, the plankton sample was strained through a coarse nylon mesh to remove large animals and placed in several insulated jars. At Woods Hole, *A. clausi* were sorted from the plankton by pipette and placed in 1-liter insulated jars, or 6-liter styrofoam jars in a concentration of about 100–150 individuals per jar. In Oregon the jars were partially full of seawater containing 37 mg liter^{-1} (10^{-4} mol) of EDTA, a complexing agent, and 6.25 mg liter^{-1} of penicillin G, an antibiotic used only during the initial period of culture (Neunes and Pongolini 1965). The jars from Woods Hole were shipped via air freight, with a transit time of 1–3 days.

Upon arrival at the laboratory in Corvallis, Oregon, the plankton samples were diluted with precooled seawater, and adult *A. clausi* were sorted into different containers. A concentration of not more than one adult copepod per 35 ml of water was allowed (Urry 1965; Corkett and Urry 1968). Individuals at all levels of development were transferred to clean dishes and sea-

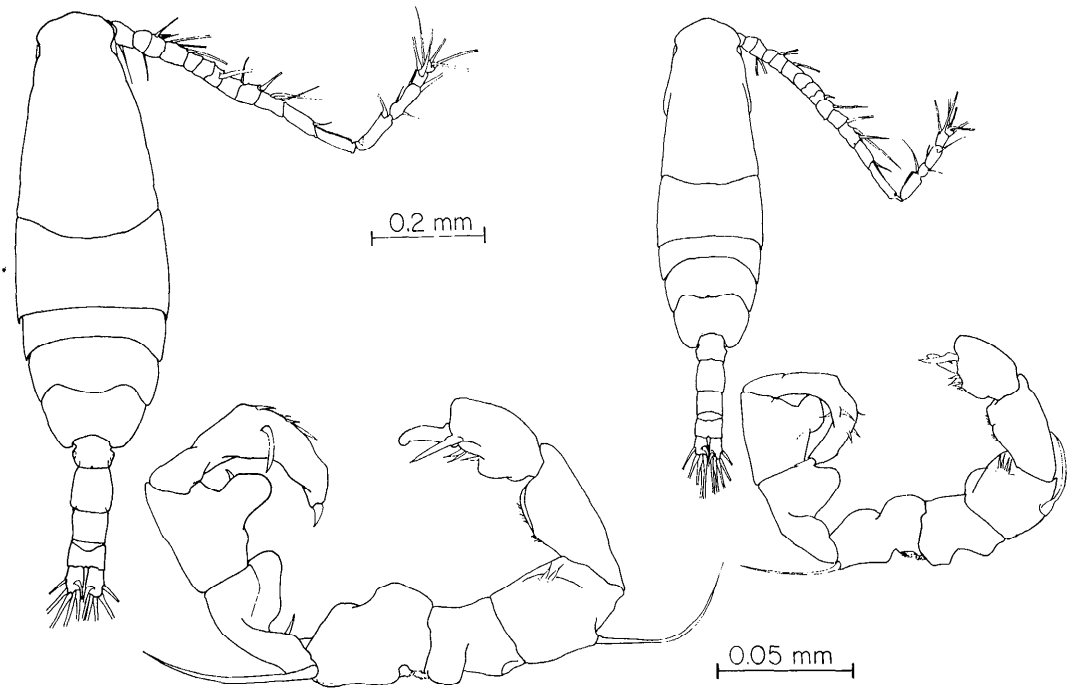

Fig. 1. Left—male of the Altantic form of *Acartia clausi* reared in culture at 17°C together with the fifth thoracic leg of a different specimen. Right—male of the Pacific form similarly reared.

water weekly to avoid excessive growth of bacteria. Most of the water was drained from each container by means of a mesh covered siphon; the remaining water and animals were poured into a petri dish, examined under a dissecting microscope, and pipetted to clean beakers. All developmental stages were kept in 500 ml of water with densities of up to 10 adults, 25 copepodites, or 100 nauplii per beaker. Copepodites for breeding experiments were individually reared to adulthood in 100 ml of water to prevent contact of males and females before the experiment. Glassware was washed with distilled water and sterilized before use, avoiding the use of any detergent.

All the containers were covered with a Speedyvap watchglass cover, designed to permit free exchange of air over the water surface. The culture room was continuously illuminated by fluorescent lamps over the stock cultures of algal food. The temperature was kept at 17°C ± 0.5°, allowing very short development times (about 24 days) convenient for the work. A mixture of the diatom *Thalassiosira nordenskioldii* Gravida in a concentration of about 5,000 cells ml^{-1}, the flagellate *Rhodomonas* sp., 30,000 cells ml^{-1}, and the chrysomonad *Isochrysis galbana* Parke, 40,000 cells ml^{-1} was fed to the copepods at each transfer. The algae were added to the copepod cultures in their original culture medium. Algal cultures were maintained by standard techniques and samples counted on hemocytometer slides. On the fourth day of the week between transfers enough food was added to maintain the initial concentration.

Pacific seawater of 32 to 33‰ salinity, containing EDTA in a concentration of 37 mg $liter^{-1}$ (Neunes and Pongolini 1965), was used in all the experiments. It was collected either in the Pacific Ocean within a few kilometers of the coast or in Yaquina Bay at high tide. The water was membrane (0.45 μm) filtered twice before use.

The interbreeding experiment was carried

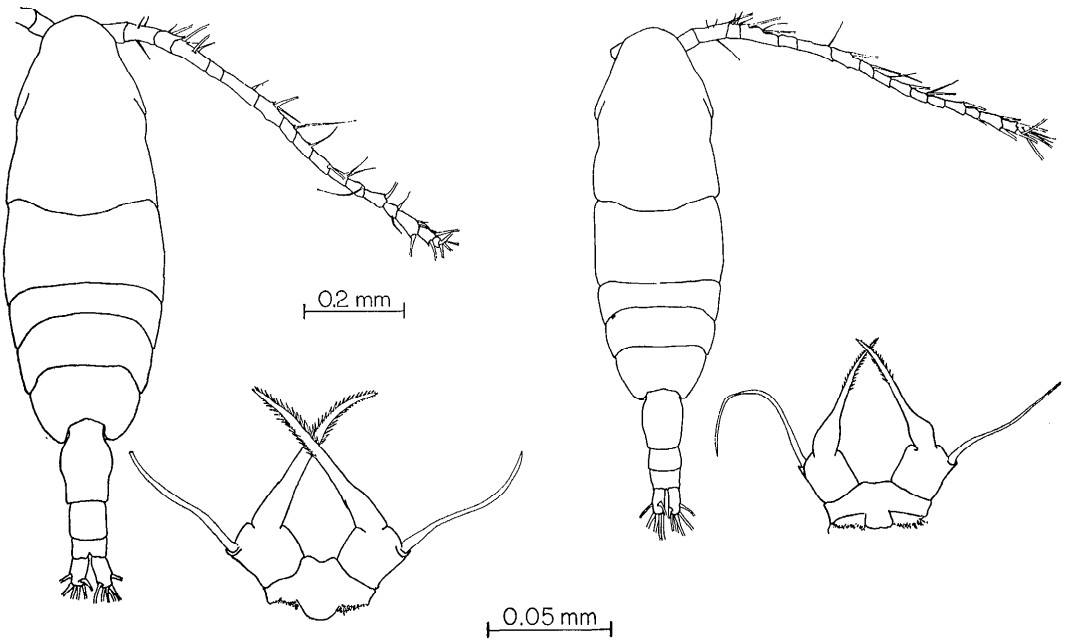

Fig. 2. Left—female of the Atlantic form of *Acartia clausi* reared in culture at 17°C together with the fifth thoracic leg of a different specimen. Right—female of the Pacific form similarly reared.

out under the conditions described for laboratory culture. Individually reared females were placed with males from the other source, some individually reared, some from stock cultures. The number of organisms per beaker depended on the availability of males and females in each culture, up to a maximum of 10 copepods per beaker. Control beakers containing females and males of the same population were set up at the time of the interbreeding experiments. After 1 week, the beakers were examined, and the presence of eggs, nauplii, and spermatophores attached to the females was recorded.

Several individually reared females from both Atlantic and Pacific sources were kept singly in 150-ml beakers to determine if unfertilized females would produce eggs, as observed by Jacobs (1961) and Corkett (1967).

Adult copepods were preserved in Formalin and mounted on slides in glycerin for comparison of important morphological characters.

Results

The results of the interbreeding experiments and the controls are summarized in Tables 1 and 2. Differences in the numbers of males and females of each population were determined by the availability of copepods at the time of each experiment. We did more experiments with Atlantic females and Pacific males than with Pacific females and Atlantic males (*see Table 1*) because it was easier to obtain males from the Pacific stock population. Nauplii or females carrying spermatophores were found on only four occasions in the interbreeding experiments. All three females found bearing spermatophores were separated to see if they would lay fertile eggs; all three died within 1 week. In both cases where live nauplii were found there was opportunity for experimental error. Most of the copepods used for these experiments came from cultures started on 22 February 1973 for the Pacific population and 6 June 1973 for the Atlantic population. However, Atlantic females from a culture started on 9

Table 3. Sizes of adult Acartia clausi *cultured at 17°C. Measurements were made with an ocular micrometer. The numbers in parentheses are 95% confidence limits for the mean:* t × SE.

Source and sex	No.	Mean total length (mm)	Mean prosome length (mm)
Pacific			
female	27	0.938 (± 0.022)	0.721 (± 0.016)
male	15	0.779 (± 0.010)	0.577 (± 0.010)
Atlantic			
female	16	1.068 (± 0.022)	0.800 (± 0.020)
male	20	0.986 (± 0.015)	0.737 (± 0.013)

March 1973 were used in the first interbreeding attempt started on 29 May 1973. These females were not individually reared, but no spermatophores or nauplii were observed for 2 weeks before the beginning of the experiment. It is possible, however, that they had been previously fecundated by Atlantic males. The Pacific males used in two of the 17–24 July experiments were also not individually reared, and it is likely that a few eggs laid by the Pacific females in the stock cultures were transferred with the males. None of the four nauplii from the experimental crosses survived an attempt to rear them.

In all cases there was successful breeding in the control experiments between individuals from the same population (Table 2). Success is defined as the production of abundant offspring and the normal development of these into a fertile adult population. The number of nauplii produced ranged from 12 for a Pacific × Pacific cross on 4 July to 107 for an Atlantic × Atlantic cross of 15 July. On 16, 22, and 23 July at least 25 nauplii were recorded for each beaker in the Atlantic population. None of these counts is a careful measure of fecundity and should not be used as such. Females carrying spermatophores were recorded at times for both Atlantic and Pacific copepods in the control beakers.

It is obvious that each population reproduces with good success under our culture conditions, but that there is no interbreeding. The Atlantic culture has at this writing (December 1973) been in continuous culture for 6 months. Apparently Atlantic seawater is not required for their survival, but it is possible that the range of acceptable breeding partners is reduced for the Atlantic population by the foreign conditions and that this is the mechanism of the failure to interbreed. Unfertilized eggs were found in all beakers containing individually reared females of both populations.

Figures 1 and 2 compare the gross morphology of males and females from each population after the Atlantic individuals were in culture for two generations, the Pacific for about six generations at 17°C. Fifth thoracic legs of both sexes are also compared. Atlantic males and females are bigger than Pacific males and females, and the size difference between the Pacific male and female is greater than between the Atlantic male and female (Table 3). The shape of the fourth segment of the male urosome is different: in the Atlantic form it bears a triangular projection pointing posteriorly on the dorsal side, in the Pacific male the projection is indented at the midline. Fifth legs of the females are very similar in relative size and morphology. The fifth legs of the males of the Atlantic form are relatively bigger than those of the Pacific form. There are no consistent differences in segment shape or armature of the male fifth leg not attributable to the size difference.

Discussion

The Atlantic and Pacific populations of *A. clausi* are geographically isolated but have been grouped for many years under the same name, based on morphological similarity (Esterly 1924). Our comparisons between cultured individuals from both populations indeed showed remarkably similar individuals, differentiated mostly by size, and by a few subtle details in the males. Mayr (1963) pointed out that most geographic isolates differ in some morphologic characters. These differences are not necessarily evidence of reproductive isolation, but are frequently correlated with it. We have shown experimentally that under the same environmental conditions, Atlantic and Pacific populations of these mor-

phologically similar copepods were able separately to breed and produce fertile offspring, but *not* when members of the two populations were brought together. Evidently, they have diverged enough to become reproductively isolated.

We do not propose that a new specific name be assigned to the Pacific population for several reasons. First, there is never confusion about the source of an individual because of the use of a name common to both populations. Second, it is not yet clear that either the Pacific or the Atlantic population of *A. clausi* is a single population with interbreeding potentially possible between all of its geographically farflung parts. The assignment of new specific names should only follow a larger, possibly worldwide, study of *A. clausi* populations. We follow in this the taxonomic philosophy of Wilson and Brown (1953).

Physiological, ecological, and behavioral differences have been found in closely related and similar species. According to Mayr (1963), this indicates that each is a separate biological system with species-specific tolerances to temperature, humidity, and other physical and biological factors. Indeed, observations on the development of both populations of *A. clausi* under the same environmental conditions showed that certain aspects of their biology were different. The Atlantic *A. clausi* had a somewhat longer developmental time, which is doubtless correlated with its larger terminal size. The Pacific population produced a higher proportion of males under the same conditions. More studies will be made to evaluate these differences. We would like to point out to ecosystem modelers, however, that it is dangerous to use results from Atlantic culture work to make ecological inferences about Pacific populations and vice versa.

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