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Medaka fish had the honor to perform the first successful vertebrate mating in space

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Abstract Among all the vertebrate species, medaka (*Oryzias latipes*) became the first species who demonstrated mating behavior in space. Moreover, the eggs they laid developed normally and hatched as baby fish in space. For a long time it has been believed that when exposed to weightlessness or microgravity (micro-G), fish exhibit looping behavior. Using the 20 seconds of micro-G realized by a parabolic flight of an airplane, we have found strains of this species which do not loop at all under micro-G. Four adult fish of the strain resistant to micro-G were sent to space. Selection of the four fish was crucial in the success of the space medaka experiment. Based on a hypothesis presented for posture control of the fish, strongly eye-dependent fish were selected. Selection procedures and what were achieved in space are described in detail. Post-flight studies on the offspring are also introduced. So far, no effects could be detected on the offspring of the four medaka fish who made a 15-day space travel aboard the space shuttle Columbia.

Introduction

In the summer of 1994, four medaka fish (*Oryzias latipes*) made a space travel of 15 days in the space shuttle Columbia. The entire mission is called the Second International Microgravity Laboratory (IML-2/STS-65). The four fish performed their mating behavior in space for the first time among vertebrate animals. Moreover, the eggs they laid developed normally till hatching fry (baby fish) in space (Ijiri, 1994). The success of the medaka experiment owes much to the selection of the four fish sent to space. Here, I will report on the idea used for selecting the final four fish and also on what the four medaka fish have achieved in space. The results of post-flight analyses on their offspring will also be presented.

Finding fish strains tolerant to microgravity

On the Earth, fish body itself can be in near microgravity (micro-G) conditions because buoyancy is balancing its weight in the water. So, everybody had thought that among vertebrates fish should be the easiest one to accomplish reproductive mating in space. In days of Skylab (1973) small fish (*Fundulus*) were sent to space, where they continuously looped for 3 days until they gradually adapted to micro-G. Between such looping fish, nobody expected mating performance at all. Even fish begin to adapt to space environment after a few days of continuous looping, they have already been exhausted from looping and eating no food, and as its consequence having no vigor at all for mating. Since then, 'Fish do loop under micro-G' has been believed, and space studies using fish have been mostly on their looping mechanism, regarding it as a model system for space motion-sickness in human.

In this IML-2 mission (1994), about 20 years after the looping behavior of fish was reported, the first successful vertebrate mating was realized using medaka fish. The main reason for its success is that we have found fish strains which do not loop in micro-G, so that they could swim normally from the first time they reached space. Such finding was accomplished using parabolic flights by a jet airplane, testing many strains we had for their tolerance for micro-G.

There are many strains in the medaka *Oryzias latipes* (Tomita, 1992). I had a time to ask to myself what a strain is. For example, when we try to establish a new (inbred) strain, we start with a pair of fish (a male and a female). Usually, such a pair to start with are chosen almost randomly from a heterogeneous population. Then, repeating sister-brother matings through many generations, if lucky to have surviving offspring still, we have established a strain (e.g., Hyodo-Taguchi and Egami, 1985). Of course, we may start with a pair of fish which have a certain trait or gene(s) of interest. Even in such a case, when selecting a starting pair it is usual to give no special considerations on the rest of genes. Thus, an established fish strain is a population of fish having an identical

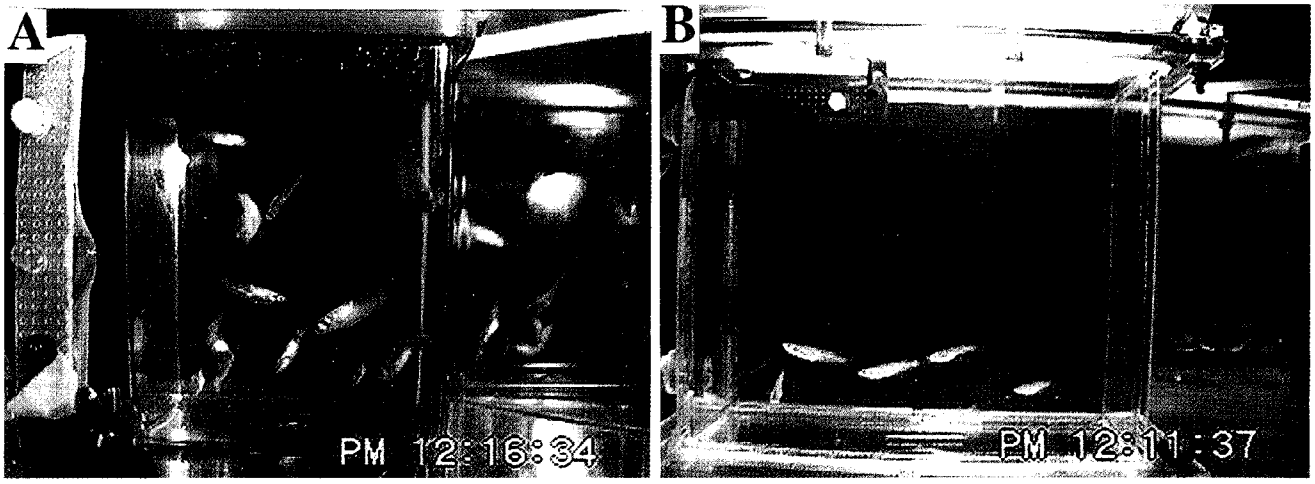

Fig. 1

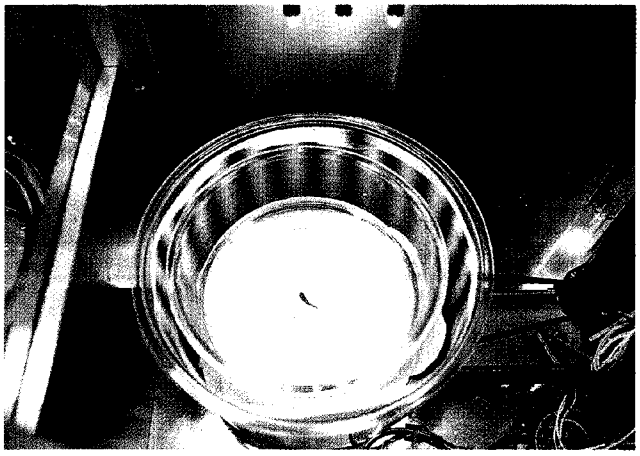

Fig. 2

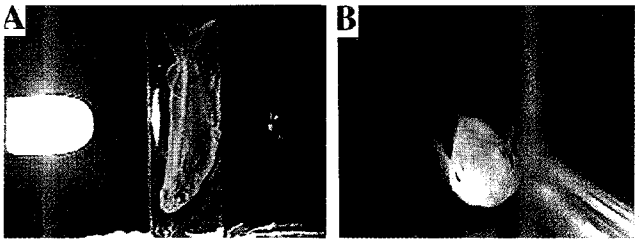

Fig. 3

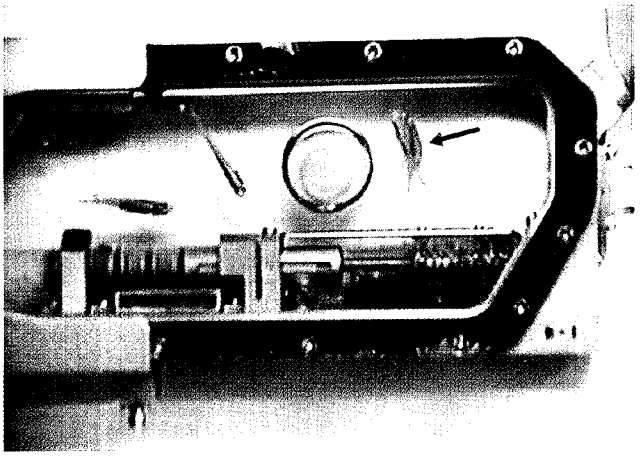

Fig. 4

Fig. 5

Fig. 6

Explanations, at the next page bottom.

genetic constitution, however, all genes of which came from one pair of fish chosen randomly from among a heterogeneous fish population.

When considering human responses to micro-G, some people are resistant or tolerant to micro-G, while others not. This can be easily understood since even among astronauts differences were reported in the degree of their space motion sickness. If so in human which is a heterogeneous population, in medaka fish there also can be ones tolerant to micro-G. If such fish had happened to be employed as a staring pair, we may already have a strain which shows tolerance for micro-G. When no such strains were found, still it is possible to establish a micro-G-tolerant new strain after finding some tolerant fish and carrying out a procedure for cloned-fish production (Ijiri, 1987).

With this idea, we proceeded to the airplane experiments. By the parabolic flights using a jet-airplane (MU-300, operated by Diamond Air Service Co. Ltd., Nagoya, Japan), micro-G of 20 seconds duration was realized, and as many strains as available were exposed to micro-G. As soon as started the experiments, we were shocked to see how rapidly the fish continue to loop in micro-G. In one strain, some fish looped at a rate of several times per second at the most rapid instance during 20 seconds of micro-G (Fig. 1A). They always loop in diving motion, i.e., looping with their belly inside. When we put such fish in the aquarium designed for keeping the medaka fish in space, some fish bumped the head on the wall of the aquarium, having a brain concussion. If such fish went to space, they all will be dead far before they adapt to micro-G, of course far before they mate.

A hopeless state did not last so long. After checking several strains, we found strains tolerant to micro-G. Under micro-G all the fish of the strains did swim normally without any loopings at all (Fig. 1B). A long-believed story that fish loop when being exposed to micro-G does not always hold true. Of course, repeating the parabolic flights many times we made sure of such behavior of the fish strains. Moreover, different generations of the strains were exposed to parabolic flights, the results demonstrating such tolerance for micro-G always inherited from parents to children, to grandchildren, and to great grandchildren.

Why particular strains of medaka fish can control their posture under micro-G? We then kept the fish in a complete darkness and observed their behavior in a parabolic flight. Now, all the fish strains looped. The fish which can control their posture under light conditions lost their ability of posture control when kept in dark. This implies the contribution of visual sense in keeping their posture under micro-G. The other strains which loop in light, of course, loop in dark with more loopings than in light.

Then, in the laboratory we tested visual acuity of medaka fish. A simple way is using a rotating striped-drum apparatus (Fig. 2), checking whether fish can follow the movement of a drum with black-and-white stripes (Ohki and Aoki, 1985; Ijiri, 1993, 1995). It is their instinct to follow the stripes. If rotated at the low speed, any fish can follow the movement of stripes. With an increase in the rotating speed of the drum, if fish can still differentiate black stripes from white ones, he will follow the rotating drum. When two colors merged into uniformly grey in the eyes of fish, he will stop tracing the drum.

Those strains of medaka fish that looped in parabolic flights soon stopped following stripes with a little increase in rotating speed, while the strains tolerant to micro-G traced the stripes perfectly even in a far more rapid rotation.

Fig. 1. Behavior of medaka fish under microgravity (micro-G) generated by a parabolic flight of airplane. **A.** Strain which exhibits looping behavior. **B.** Strain tolerant to micro-G. All the fish are swimming normally without any loopings under micro-G.

Fig. 2. A rotating striped-drum apparatus to check the visual acuity of fish. Why do fish follow the rotating direction of stripes? Suppose they are in a stream with a water-flow. They usually are staying at a certain point with their heads toward upstream. They may see a stake, water plants or stones (these correspond to the stripes). Due to the water-flow the fish are drifted backward, which may look as if the stake is moving upstream when relatively thought that the fish are staying still. On instinct, following the stake the fish move upstream. With this movement, the fish can stay at a certain point in a stream.

Fig. 3. Dorsal-light reaction tests. **A.** Method I. The lamps repeat lighting in turn. **B.** Method II. The light always comes from the left, and degree of slanting to the light is measured. For details, see text.

Fig. 4. Mating behavior of medaka fish in space (arrowed). Male is embracing female using his fins. The first time reported in vertebrates.

Fig. 5. Many eggs laid in space have developed normally, showing two black-pigmented eyes.

Fig. 6. A hatched fry (arrowed) swimming with a mother fish.

A hypothesis to explain the strain-difference in the fish behavior under micro-G

Man can control his posture with both visual and vestibular informations, integrating these in the brain. So are medaka fish. With a vision, the fish can recognize the scene around him and control his posture. In the vestibular system, the otolith is pulled down by the gravity force, the gravity (acceleration) is transmitted to the sensory haired-cells, and the direction of gravity can be recognized in the brain. Fish swim orienting their belly side to the direction of gravity. Fish, however, use another powerful direction-sensing mechanism, i.e., dorsal-light reaction. This is a reflex that fish turns his dorsal side to the light source, i.e., turns his back to the direction from which light comes. Therefore, in fish his dorso-ventral axis is determined both by the direction of gravity and by the direction of light, though on the Earth the contribution of gravity is extremely larger than light.

In medaka fish, we have proved that there exists a clear strain-difference in the ability to swim under micro-G. Most strains do loop, but there are some strains which can swim normally. The following is my hypothesis to explain why such strain-differences exist in their response to micro-G.

Let us consider a situation on the Earth. Some strains of medaka have a better vision than other strains, for example, the strains which can follow easily the high speed of rotating stripes are the case. Such superiority in visual acuity of a strain is a talented nature, which is an overall outcome of many genes expressed. A high visual acuity means they are also sensitive to light. These fish may use scenery informations and light-direction more for controlling their posture, therefore depend less on the gravity (i.e., less on the otolith system) than other strains. Such fish may be typed as 'strongly eye-dependent'. There may be another way of thinking for that strain. The strain might have a trouble in the gravity-sensing organ (i.e., vestibular function) or in the integration of the gravity information in the brain. In such a case, they also depend strongly on visual informations.

A completely opposite type of fish may exist. Another strain of fish may have a low visual-acuity, therefore depend more on the otolith information. Or they may have a good efficiency in vestibular function, being a 'strongly gravity-dependent' type.

Each fish strain can be typed either as an eye-dependent strain or as a gravity-dependent one, and which type the strain belongs to is determined by their genetic constitution. So far as the fish are swimming on the Earth, whichever type they are, they probably have never experienced much difficulty in controlling their posture. Unexpectedly, they are now put in the airplane, and are exposed to micro-G, where the otolith system does not help the fish at all. It rather disturbs their posture-control mechanism, sending strange informations as ever experienced. Therefore, the fish continuously keep looping in such confusion. The fish strains that can swim calmly or being tolerant to such micro-G situation, are those who have been swimming routinely depending much on scenes and light-direction. Thus, in micro-G, strains of gravity-dependent type do loop and eye-dependent strains can swim normally.

Such is a hypothesis I finally came to after considering the strain-differences in behavioral response of medaka fish to micro-G. The test methods employed for selecting the fish sent to space were mostly based on this hypothesis.

Selection procedures for the four fish sent to space

Strain and individual difference in medaka fish

Repeating more parabolic flights, we found in total, two strains tolerant to micro-G. They were ccT strain and HNI-II strain. In the laboratory, we repeated the ground experiment many times keeping the fish inside the aquarium of space-use for two weeks. The strain HNI-II was found nervous in nature and very sensitive to various stresses. In many cases, the fish of HNI-II strain stopped mating and laying eggs when being placed into the aquarium. For this reason, use of HNI-II strain was withdrawn, and ccT which is a fairly resistant strain to stresses was chosen. The ccT strain is orange-red in color.

Now, the strain to be used in space has been decided. It was however not the end, rather it was a start of the actual selection. Since we can send only four fish (two for each sex) to space, the further effort should be concentrated much on selecting the most tolerant ones to micro-G from among the fish population of this particular strain. As a general rule, there still exist individual differences even among the population of a totally identical genetic constitution. Such individual difference in a certain character is generally called 'environmental variation', and not heritable. With all genes being identical, such non-heritable quantitative character (for example, degree in tolerance

for micro-G) shall show a certain distribution in its quantitative measure. Even when we have picked fish in the top population on that distribution and produced their children, the quantitative character of these children will take the same distribution, with no change in its parameters like the mean and the variance. Thus, for selecting the fish-astronauts from among the fish population of ccT strain, we have to test each individual fish for its tolerance for micro-G.

Selection methods employed

The hypothesis drawn above on the tolerance for micro-G was the only one I could rely on at that time. Although it was a hypothesis based on the data in parabolic flights, i.e., in micro-G of 20 seconds, I could not find any other theories to convince me scientifically. What the hypothesis tells us is to select the fish 'strongly eye-dependent,' and send them to space. For this, three selection methods, i.e., a), b) and c) listed below were employed.

a) Ability to follow the rotating striped-drum

The test is to choose an individual fish with a prominent visual acuity. As explained earlier, the ability to trace the moving stripes was tested (Fig. 2). In the actual selection, the test was performed in a severe condition. The drum was rotated at a high speed of 72.5 cm/sec (261°/sec) under the dim light (20 lx at the fish). From external appearance and size, 1500 fish were firstly chosen out of 2000 fish of ccT strain, and such 1500 fish were given the test in this rotating striped-drum apparatus, resulting about 60% of the fish passed the test.

b) Dorsal-light reaction test (method I)

We employed two different methods (methods I and II) for testing the ability of dorsal-light reaction in medaka fish. Dorsal-light reaction is a reflex most fish species have, turning the dorsal side to the light source. In the method I, each fish was put into a test-tube with water, and the lamps at both sides of the tube were lighted in turn (Fig. 3A). The time each fish takes to turn his dorsal side to a newly switched light-direction (reaction time) was measured. The method was applied to each of about 900 fish who passed the rotating striped-drum test [see a), above]. The test was carried out only in Japan. A fish was judged as 'passed the test' when he turned his dorsal side to the lamp within 30 seconds after the lamp was switched on. The fish that did not respond at all despite 10 times of switching or those took more than 30 seconds in response were removed. The method I has a merit of taking a short time for each fish. In other words, you cannot keep the fish inside the test-tube more than 10 minutes without damaging them.

c) Dorsal-light reaction test (method II)

After transferred the 300 selected fish to USA, i.e., to Kennedy Space Center (KSC), Florida, the method II was employed for checking their degree of dorsal-light reaction. It was difficult to apply this method to all of the 900 fish in Japan because the method takes much longer time for each fish than method I. Between method I and method II there is a slight difference in what nature of the fish the test was focusing on. In method I, the tail-head axis of fish is the same direction as of gravity, and the gravity does not determine which direction he puts his dorso-ventral axis to. On the contrary, the method II checks the degree of his slanting to the light, thus measuring his dependence on light relative to his dependence on gravity (Fig. 3B). In this test, we aimed at selecting the fish who show a high dependence on light, i.e., strongly eye-dependent fish. At KSC, only the fish still remaining healthy were given this test.

Since on orbit fish cannot depend on gravity for their posture-control, we decided to provide an oriented lighting for helping their posture-control, i.e., expecting fish to use this reflex of dorsal-light reaction and always turn his back (dorsal side) to the light source. In space, to the fish aquarium, lighting was set so that it comes always from one side.

Space-shuttle experiment

Four fish (two males and two females) were put in the aquarium. The aquarium was then connected to the AAEU (Aquatic Animal Experiment Unit), which supplies water oxygenated and biologically filtered. Fish were kept under temperature- and light-controlled conditions (24°C, 14-h light and 10-h dark). Every 3 days the crew sent the new food to the accessible position in the food-supplying apparatus. Since the fish had been mating everyday before loaded to the Shuttle, if not suffered much damage in micro-G, the fish may mate and lay eggs also in space. Each day, mating behavior should be completed within 2 hours after the transition from dark period to light period. The crew checked the existence of newly-

laid eggs every day. On the 3rd, 5th and 8th day of the mission, the video camera recorded their activity for two hours immediately after lighting, and this was an enough time to record the fish mating behavior.

Newly-laid eggs first form a cluster on the belly of the female fish, then after an hour or so, they leave the body. Eggs detached from the female body flow with the water to an area separated by a mesh structure. This area is called 'children's room'. The crew carried out close-up video observations of the developing embryos and hatched fry. All the adult fish, fry and embryos returned safely to the Earth.

Following are events in the IML-2 medaka experiment, listed in a time-course order.

1) Adult fish (2 males and 2 females, of orange-red colored type, ccT strain) were put in the aquarium. These four fish were named as 'Cosmo' (as meant in English) and 'Genki' (meaning 'active' in Japanese) for males, 'Yume' (dream) and 'Miki' (future) for females. These names had been proposed to us by boys and girls of Young Astronaut Club in Japan. The medaka aquarium was loaded into the Spacelab about 30 hours before the launch, and space shuttle Columbia had a lift off just on a scheduled time. When examined at 9 hours after the launch, no eggs were detected. Then, about 24 hours after the launch, our Japanese payload specialist, Chiaki Mukai reported 3 newly-laid eggs by her visual inspection.

   In the fish medaka, mating and laying eggs and fertilization of eggs all take place almost at the same time. Therefore, from her report we could tell that male and female fish did mate and laid eggs in space. The spawning habit of this fish is, once they started mating they continue to repeat mating and laying eggs once every day. So, as expected, on the next day again they mated and laid eggs in space, and Chiaki Mukai reported number of eggs increased to a total of 10 in the aquarium.
2) On the 3rd day of the mission, the crew (Donald Thomas) set up the video-camera and tried to record the mating behavior of the fish. And again, male and female fish mated (Fig. 4). Lucky enough, they did mate in the midst of video down-link time, so that I could see with my own eyes this dramatic scene, not waiting the arrival of recorded video-tapes after the landing of shuttle.
3) Crew reported the existence of many eggs in the aquarium and most of these had well-developed body with pigmented eyes. On the 5th day, mating behavior was again video-recorded, and crew (Richard Hieb) took a good video-picture of a female fish with eggs at her belly. Most eggs seemed developing normally (Fig. 5), and for those eggs laid on the early days of the mission baby fish (hatched fry) were expected. Usually at 24°C, fertilized eggs will become hatched fry about 10 days later. However, due to the trouble (death) in newt aquarium, water temperature has been lowered to 23°C, which may have somewhat prolonged the time of hatching. At the end of 12th day, a baby fish was detected by Chiaki Mukai when she carried out a routine visual-inspection. Video-recording is not scheduled on this day. However, she decided to take a video picture of that swimming baby. With the help of Richard Hieb, she successfully video-recorded the picture of a baby fish swimming with a mother fish (Fig. 6). On that evening, the picture was broadcasted as crew's edition for NASA's mission high-light TV program.
4) The shuttle extended its stay in space with one day, and landed on KSC in the morning of July 23. After watched its landing, we were waiting for a NASA's vehicle to bring the medaka aquarium to KSC laboratory (Hangar-L). When four adult fish were lying down at the bottom of the aquarium, we all were shocked. The fish looked as dead. However, checking their fins and gills were moving all right, we soon understood that they were only having a trouble with the gravity force on the Earth. Then one by one, fish began to swim or tried to swim. Though quite awkward they swam, a smile came back on our face.

   Another surprise was that eight baby fish were detected in the area separated with a mesh-plate. An extra day of extended flight-duration may have brought about this increase in the number of fry hatched in space. In total, 43 eggs were laid (detected) in space, out of which 8 baby fish were born (hatched as fry) in space. 30 fry hatched after landing. 5 eggs out of 43 stopped their development at early developmental stages or they might have been unfertilized. This is the normal hatching rate, comparing with the ground-based or laboratory data.

   Adult fish returned from space had forgotten

how to swim in the world of gravity force. In space, for about 15 days they did not have to use air bladder (swim bladder) at all, and had forgotten how to use it properly. On the Earth, fish tried to swim upward, however, after a second or so they dropped again onto the bottom of the aquarium. They were also swimming without much use of tail-fin. This probably was because the fish had not used their tail-fin in space so frequently as they usually do on the Earth. On the contrary, eight baby fish which hatched in space from the eggs laid in space (i.e., truly space-originated fry) could swim normally showing no difference in their behavior at all from that of ground-kept fry.

5) It took fully 3 days for the adult fish to recall how to swim on the Earth. From the 4th day after landing, adult fish swam normally. A week after landing (July 30), we came back to Japan with the fish. In our lab, we paired Genki (male) and Yume (female) and put them together in an aquarium, and paired Cosmo (male) and Miki (female) in another aquarium. Both pairs mated and laid eggs in the evening of the arrival to the lab (July 30) or at the corresponding time of the morning of USA Eastern time. For each pair, eggs have been collected everyday, and their development was traced till hatching.

Post-flight analysis

In space, medaka fish mated and laid eggs. Thinking of the fact they mated from the very first day of the mission, for the fish it seemed not a very hard job to live under micro-G. However, post-flight analyses on video-tapes have revealed details on their life in space, of which results may suggest that they had a hard time in mating, and also had suffered stresses due to being exposed to micro-G. The details on this subject are given in the book recently published by the author (see *Note added to the proof*, at the end of this paper).

Effects on offspring

As reported above, the adult fish re-started mating and laying eggs on the 7th day after landing and continued to do so everyday afterward. The fry hatched from these eggs laid after returning to the Earth (space medaka 2nd generation) were reared to grown-ups in our lab, and distributed to various places in Japan. Out of more than 5,000 proposals for adopting these fish children, so far 304 places have received the fish. Most of them were elementary schools and school children themselves. Nice names were given to such adopted fish, and we have been receiving many thank-you letters and reports on the fish. In Japan, most textbooks for the 5th grade of elementary school are dealing with medaka fish, and pupils actually feed them and observe mating and also development of eggs laid. In some schools, they now have been using the 3rd- or even 4th-generations of space medaka in such biology classes.

Among the 8 truly space-originated fry, 4 fry were killed for histological observations. Two fry were lost when staying at KSC in natural death as is usual for these fish which lay many eggs. The remaining two fry have become adult in our lab and luckily they are a male and a female, and are laying eggs. These fish are space-originated, or so to speak, Adam and Eve came from space. This means that far before we make the Earth-originated medaka fish proliferate in Space station, such an alien fish couple have already started to create their colony on the Earth.

Figure 7 is a summary of the four adult fish traveled in space and their offspring. Space-flown adult fish re-started mating and laying eggs in our lab from July 30, 1994 (the 22nd day after the launch). Two couples were made, and each couple (a male and a female) were kept in a separate aquarium. For each aquarium, eggs laid were collected on each day. These eggs were transferred to a small glass-vessel, checking whether fertilized or not, and tracing their development until hatching fry. The data of fertilization and hatchability for the eggs laid between Genki (male) and Yume (female) are given in Fig. 8. The abscissa in the figure is given in days after the launch. That is, day 0 is the day these adult fish and also the germ cells inside their gonads were exposed to micro-G for the first time, and day 15 is when micro-G exposure was terminated. The 22nd day after the launch corresponds to July 30 when the fish re-started laying eggs.

The eggs were collected ranging 40 days (i.e., till 60 days after the launch). This was an enough range covering the complete sequence for primitive (least differentiated) germ cells to develop to fully mature germ cells, i.e., ova and sperm. In medaka fish, it takes two weeks for primitive oocytes to become mature ova, and about 30 days for spermatogonia to become sperm (Egami and Hyodo-Taguchi, 1967). Therefore, in either oogenesis (in female) or spermatogenesis (in

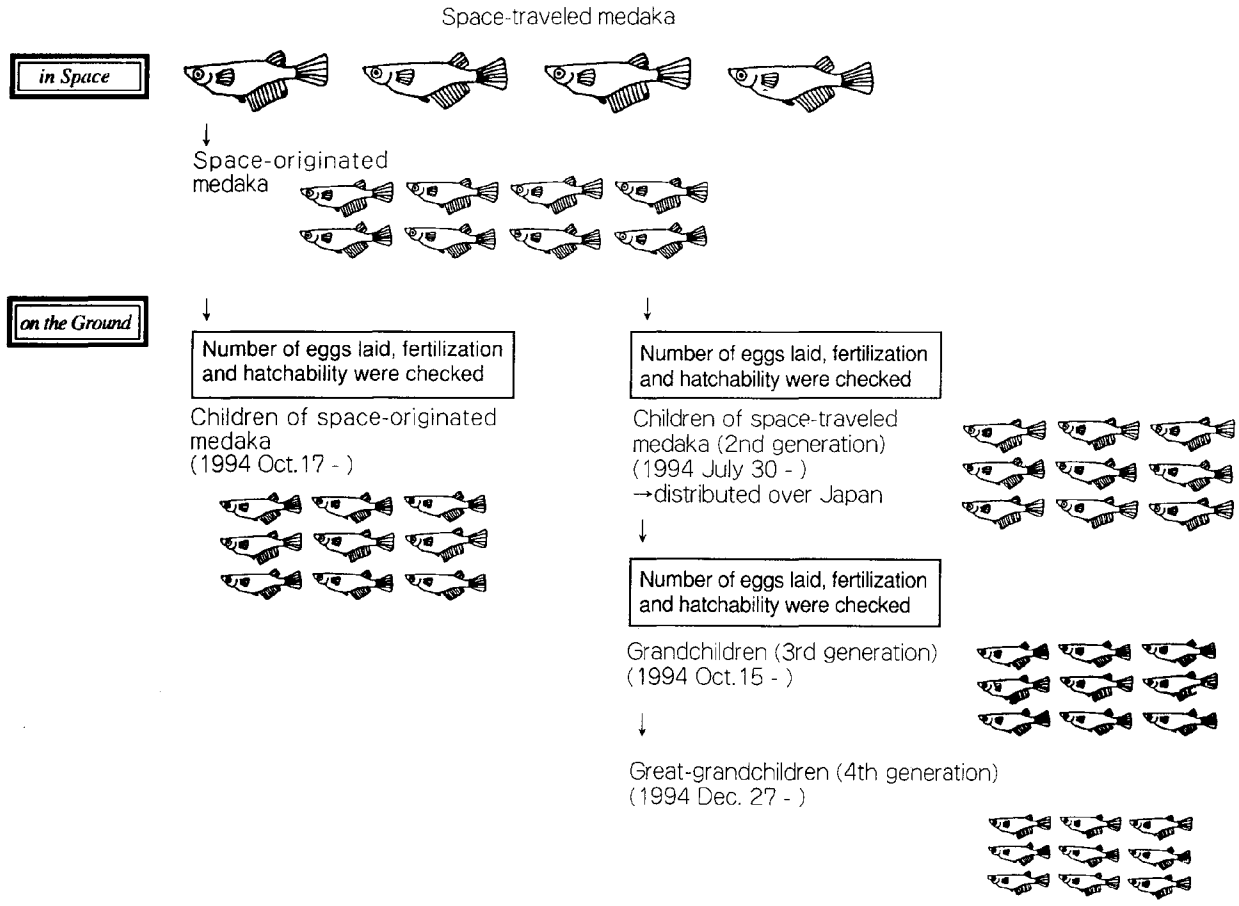

Fig. 7. Space-traveled adult fish and their offspring. The date in parentheses shows when each generation was produced for the first time, i.e., the first day their parent generation started to lay eggs.

male), if there existed some certain stages of germ cell development affected by the exposure to micro-G, within 30 days after landing we could have observed a certain period of days characterized by an extremely low percentage of fertilization or by severe effects on embryonic development thus leading to a low percentage of hatching. In Fig. 8 and in the data for the other pair (not shown here, see the book introduced at the end of this paper), no numerical differences were noted in fertilization or in hatching when compared to the eggs laid by laboratory-kept adult fish. Fish continued mating and laying eggs in the first week of the mission, and those eggs developed normally in space. This fact means that the late stages in gametogenesis (i.e., stages within a week before fertilization) were not affected by micro-G at all.

The test method we have used here is often called 'dominant lethal mutation test' and believed to detect the occurrence of large-scale aberrations on chromosomes in germ cells, which bring about non-fertilization or early death of embryos before hatching as fry. So far, the 15-day space travel has given no influences at all on the germ-cell formation process. However, the test method is not useful for detecting minute changes of genes (i.e., point mutations). Whether such mutations have been induced additionally due to micro-G, radiations and other stresses during the space flight, cannot be answered because of the small number (four) of fish and of the limitations of the test method employed.

In our laboratory, effect studies on the offspring have been extended further to the grandchildren of the space-traveled adult fish. Additionally we are now collecting data from those locations where the babies of the adult fish have been distributed to. A summary of such data will be presented elsewhere.

As illustrated in Fig. 7, truly 'space-originated fry' have also grown up and started laying eggs. The data on the embryonic development of their eggs have now been accumulated. So far, no effects can be detected on the offspring of the space-originated fish. These are the fish that experienced micro-G conditions from the stage before fertilization till at least hatching. It is known that in this fish species, the primordial germ cells have formed and migrated into the genital ridge (i.e., the future ovary or testes) already at the embryonic stages before hatching (Ijiri and Egami, 1977;

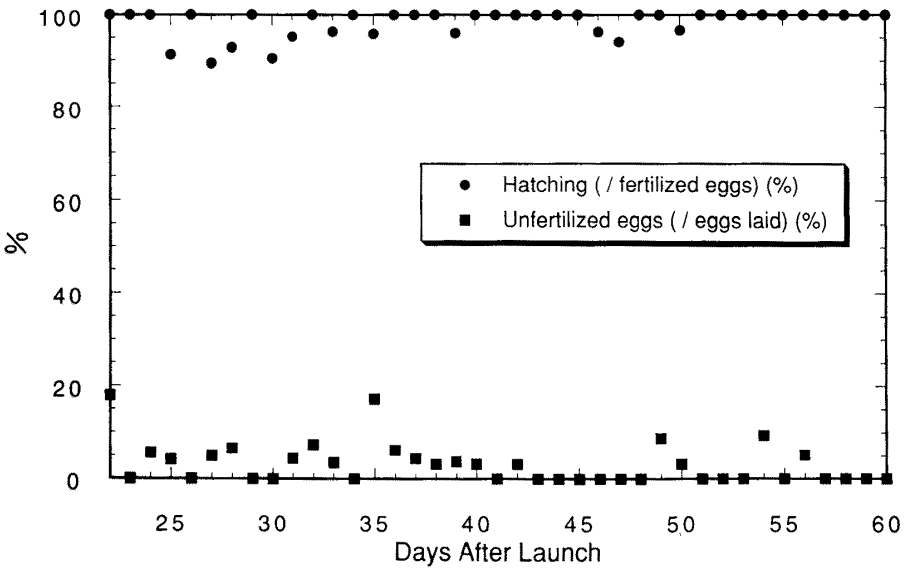

Fig. 8. Fertilization (expressed in the percentage of unfertilized eggs) and hatchability of eggs laid by space-flown adult fish (a pair of Genki and Yume) after recovery to the Earth. The abscissa is expressed in days after the launch, i.e., days after when their gonads were exposed to micro-G for the first time. Micro-G conditions ended on day 15.

Hamaguchi, 1982). Therefore, as embryos developed normally in their external appearance, inside the embryos the formation of germ cells took place normally in space, and their migration to the genital ridge was not hindered by micro-G.

Conclusion from the space medaka experiment

The present space experiment proved that fish can mate and lay eggs, and the eggs can develop normally (at least till hatching or coming out as a baby fish) in micro-G. Among vertebrate species, this was the first time in space for a successful mating between a male and a female. Of course, on the ground (1 *g*) the fish can mate and lay eggs, and eggs develop normally. Therefore, it is quite reasonable to suppose at least in the gravity range of 0 *g* (micro-G) to 1.0 *g*, fish mating and embryonic development are possible. For example, we can breed fish on the Lunar base (1/6 *g*), or when the gravity-generating space-colony is realized in future, fish-culture can be carried out at any places from the center (0 *g*) to the periphery (1 *g*) of the colony. For the four adult fish, no space-flight effects have been detected on their germ cells when offspring studies were carried out on the eggs laid in space or on the eggs laid after their return to the Earth.

Toward the Space-station experiment

The present experiment is a preparatory one for the future medaka-experiments in Space station. The IML-2 medaka experiment demonstrated that mating, laying eggs and embryonic development are all possible in space. These results showed the possibility of the multi-generation breeding of this fish in Space station, where a long-term experiment can be planned. In medaka, eggs become sexually mature fish to lay eggs within 3 months in the laboratory. They are one of the shortest life-cycle vertebrate animals. Suppose men and women have their children at the age of 20 years. Then, medaka fish can repeat its life-cycle at the speed of about 80 times of human. Using this fish system, we can study what kinds of changes might occur in the human race who have lived and repeated many generations in space. It may serve as a simulation study of human colonization in space and its consequence, of which future can be estimated 80 times earlier than the actual human case. Until when the mating in rats or mice can be realized in space experiment, the fish medaka will serve as a powerful vertebrate species in the challenge for the multi-generation experiments in space.

In the present experiment, a special place, so called 'children's room' was set inside the medaka aquarium in order to keep embryos and fry safe from adult fish. Checking whether this mechanism works or not in space was one of the objectives in this IML-2 medaka experiment. We saw many eggs drifted to there (in video images, too) and 8 fry hatched in space were also staying there safe (not eaten by adult). Thus, the separation set-ups, in most cases, worked out nicely. In Space station,

we can take out this children's room, place it into a new aquarium. Then, the next-generation fish can be reared to adult in this new aquarium. In principle, repetition of such a step will lead us to multi-generation breeding of this fish in space.

What attracts me most in the space biology experiment is the adaptation of life to micro-G. With a history of no experience of micro-G at all for about 4 billion years after the origin of life, how the medaka fish may exhibit adaptation to space when they have repeated generations in this new environment. Which gene(s) on their DNA the fish may utilize for such adaptation? This is one of the reasons why instead of artificial insemination, I have been especially concerned with their mating or their natural way of reproduction. Only the fish who have adapted well to micro-G can mate and reproduce their offspring. If that happened through a genetic change, after tens of generations such space medaka population might be somewhat different from the ground-kept populations. It might be a superb excitement to see such an experimental demonstration of one-cut scene of the evolutional process — a dream of myself as a biologist.

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Note added to the proof — After submitting this manuscript, the entire report of IML-2 space medaka experiment was published in a book titled 'The First Vertebrate Mating in Space – A Fish Story' (by K. Ijiri, RICUT, Tokyo, 1995). It has 57 pages and many pictures in full colors. The book is available free of charge on request to the author (K. Ijiri). Request can be done to his postal address, or FAX (+81-3-3816-0422) or E-mail (umedaka@hongo.ecc.u-tokyo.ac.jp).