F-3.3 Trend analysis of inbreeding depression system using inbreeding line of Japanese quail (Final Report)

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Abstract

To save the endemic, rare or endangered species of wild animals, it is essential to exploit an inbred strain of experimental animal. In the Japanese quail (*Coturnix japonica*) selected for high (H2) or low (L2) antibody production to Newcastle disease virus (NDV), a trend analysis about inbreeding depression was carried out by their reproduction record over 50 generations.

As a result, the reproductive traits of H_2 and L_2 quails changed as follows: 1) There was no trend in the egg fertility and pipping death rate throughout 50 generations, 2) The hatchability declined with the progress of generation, 3) However, the hatchability of L_2 quails was improved after the crisis of bottle neck effect happened at 43rd generation.

After crossbreeding of H2 and L2 lines, the heterosis effect appeared in the hatchability. As this heterosis recognized only few pairs, the nicking between sire and dam might be important in the inbred lines.

By means of artificial insemination, we succeeded in freezing of quail's sperm in a medium. Therefore, it is possible to get fertile eggs from the Japanese quail in accordance with chicken's method.

These analysis of those inbred Japanese quails would be useful to clarify the mechanism of inbreeding depression for rare avian species.

1. Introduction

There are 27 kinds of endangered species and 27 vulnerable species among 536 species of birds living in Japan. So far 13 species of birds have been extinguished. However, no technology applicable for conservation has been developed to the threatened avian species.

The purpose of this study is to clarify the inbreeding depression, which was shown in the extinction animal as the decline of population, using the inbreeding animal. By the way, the Japanese quail has been exploited as a model animal to overcome the inbreeding depression, which appears more severe in avian species than mammalian.

In this study, we show the trend analysis of some reproductive traits in the Japanese quail (H2 and L2 lines) over 50 generations. These analysis would be useful to conserve the rare wild animals, and to survive the endangered species by means of biotechnology.

2. Research Objective

To save the endemic, rare or endangered species of wild animals, it is essential to exploit an inbred strain which shows the inbreeding depression in reproductive traits. As a experimental animal of avian species, the Japanese quail is as useful as mouse or rat. However, it is difficult for the Japanese quail to be exploited an inbred strain by sib mating like mouse or rat.

In the National Institute for Environmental Studies (NIES), there is the Japanese quail

selected for high or low antibody production to Newcastle disease virus through 50 generations. These quails (H2 and L2 lines) have been bred by rotational cross to avoid the rapid inbreeding depression such as sib mating. The H2 and L2 quails have marked genetic background over 50 generations because of their family identification, and have escaped from endangered crisis during 20 year's selection.

By trend analysis of reproductive traits in H2 and L2 quails, we hope the inbreeding depression would be cleared clearly. And, we can find an action plan to save the endangered avian species, using H2 and L2 quails for experimental animals.

Finally, the purpose of this study is to propose how to overcome the inbreeding depression in avian species, using the inbred Japanese quail.

3. Research Method.

(1) Making database and analyzing reproductive traits in H2 and L2 quails.

In this trend analysis, H2 and L2 quails were used as pilot animals showing inbreeding depression. These quails have been selected for high and low antibody production to NDV inactivated vaccine. This selection experiment was started at Tohoku University in 1975, and these quails were introduced to the Institute for Environmental Studies (NIES) at 10th generation in 1980.

For computer analysis through 50 generations, the reproductive traits such as egg production rate, fertility, hatchability, rearing rate at 7 week's old and embryonic death rate were inputted to our original program. That is, each traits was calculated as the average of each family data, and combined with lines and generation as a web.

To estimate the long-term change of reproduction, the fitness index was obtained in each line as follows: Egg production rate x fertility x hatchability x rearing rate. If there were significant change in the fitness index, all the reproductive traits before and behind the generation were examined within family and line in accordance with the simulation.

(2) Crossbreeding between H2 and L2 quails to recover their reproductive ability

During the selection, reproductive ability of H2 quails declined more severely than that of L2 ones. Therefore, crossbreeding between H2 male and L2 female was carried out at 49th generation (Total 10 pairs). The reproductive traits such as fertility, hatchability and embryonic death rate were also analyzed by the program.

(3) Freezing of sperms in L2 male quails

It is necessary for H2 quails to avoid the bad nicking (struggle), when male (Sire) and female (Dam) are crossed for next generation. The artificial insemination is quite effective to avoid the struggle in crossing, and also to be used beyond the generation . Therefore, we tried to freeze quail's sperm into a medium and survey the activity after freezing (-150 c) and melting (38 c).

The experimental animal was supplied from L2 male quails at 49th generation. The sperm was collected from *ductus deferens* and stored in TYH medium at room temperature . The activity of sperm was evaluated as survival rate and mobility in accordance with chicken' s case. When the sperm was frozen in straw tube at -150 c (Liquid nitrogen, LNG), the activity of sperm was measured in incubator (38 c) as follows: After melting the sperm in straw tube, TYH medium was added to as equal volume. And the survival rate and mobility were evaluated in the same way.

4. Results and Discussion

(1) Changes of reproductive traits in H2 and L2 quails

Fig. 1 shows the changes of reproductive traits in H2 and L2 quails during 50 generations. As the selection progressed, the hatchability declined both in H2 and L2 lines. However, there was no trend in fertility and piping death rate in both lines. The decline of hatchability suggests a inbreeding depression as reported in previous paper, because the 0-16 day's embryonic death rate increased gradually in both H2 and L2 lines until 38th generation . After then, hatchability in L2 quails was significantly improved

after 43rd generation ($P<0.05$). This is suggested the lethal gene might be excluded in L2 quails at that time. Therefore, it is necessary to certify which lethal gene was excluded in L2 quail's chromosome by comparing the DNA analysis of electrophoresis.

Fig. 2 shows the changes of embryonic death rate in H2 and L2 quails. The embryonic death rate from 0 to 16 days increased in both lines until 38th generation. However, this was improved in L2 line from 40th generation. This shows the development difference between H2 and L2 lines caused by inbreeding depression. Further study should be done about lethal gene, adaptation gene using the various inbred quails.

Fig. 3 shows the change of fitness index in H2 and L2 quails. At 10th and 38 th generations, the fitness index declined remarkably in both lines. Not only the fitness index, but also other reproductive traits such as egg production rate, fertility, hatchability and rearing rate declined at that time. Therefore, it is quite important for inbreeding quails to maintain with skillful personnel, in addition to Good Laboratory Practice (GLP).

(2) Recovery of reproduction by cross-bred between H2 and L2 quails

When H2 male and L2 female quails were cross-bred among 10 pairs, only 3 pairs showed the heterosis of hatchability. This means that there is a nicking between H2 and L2 lines at 49th generation. At that time, inbreeding depression might be superior to heterosis effect except few pairs. In the case of endangered avian species, such good pairing become important.

In chicken, the nicking is used to produce a high quality progeny. The chick is produced by cross-bred of 3 or 4 inbred lines. Further experiments should be done to certify the heterosis
effect in the Japanese quail, as shown in the chicken.

(3) Recovery of reproduction by artificial insemination

We succeeded in recovery of active sperm of L2 quail by means of surgical treatment of *ductus deferens*. The activity of L2 quail's sperm was about 50% in TYH medium. But, this decreased about 10% after freezing in the same medium. It is suggested that there is a possibility to get fertile eggs by artificial insemination in the Japanese quail . We would like to improve TYH medium for getting activity of quail's sperm.

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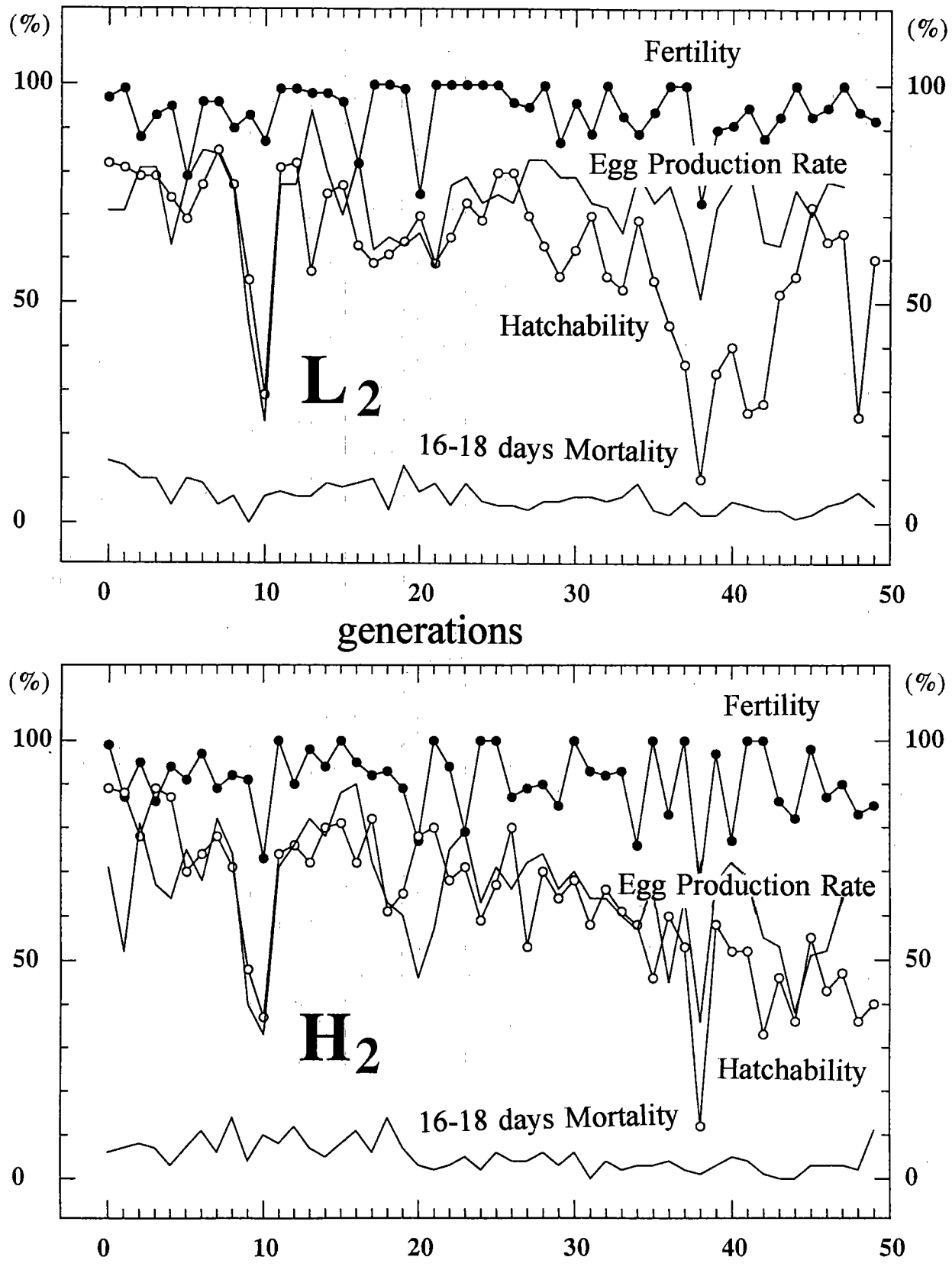

Fig.1. Changes of Reproductive Traits in H_2 and L_2 Quails

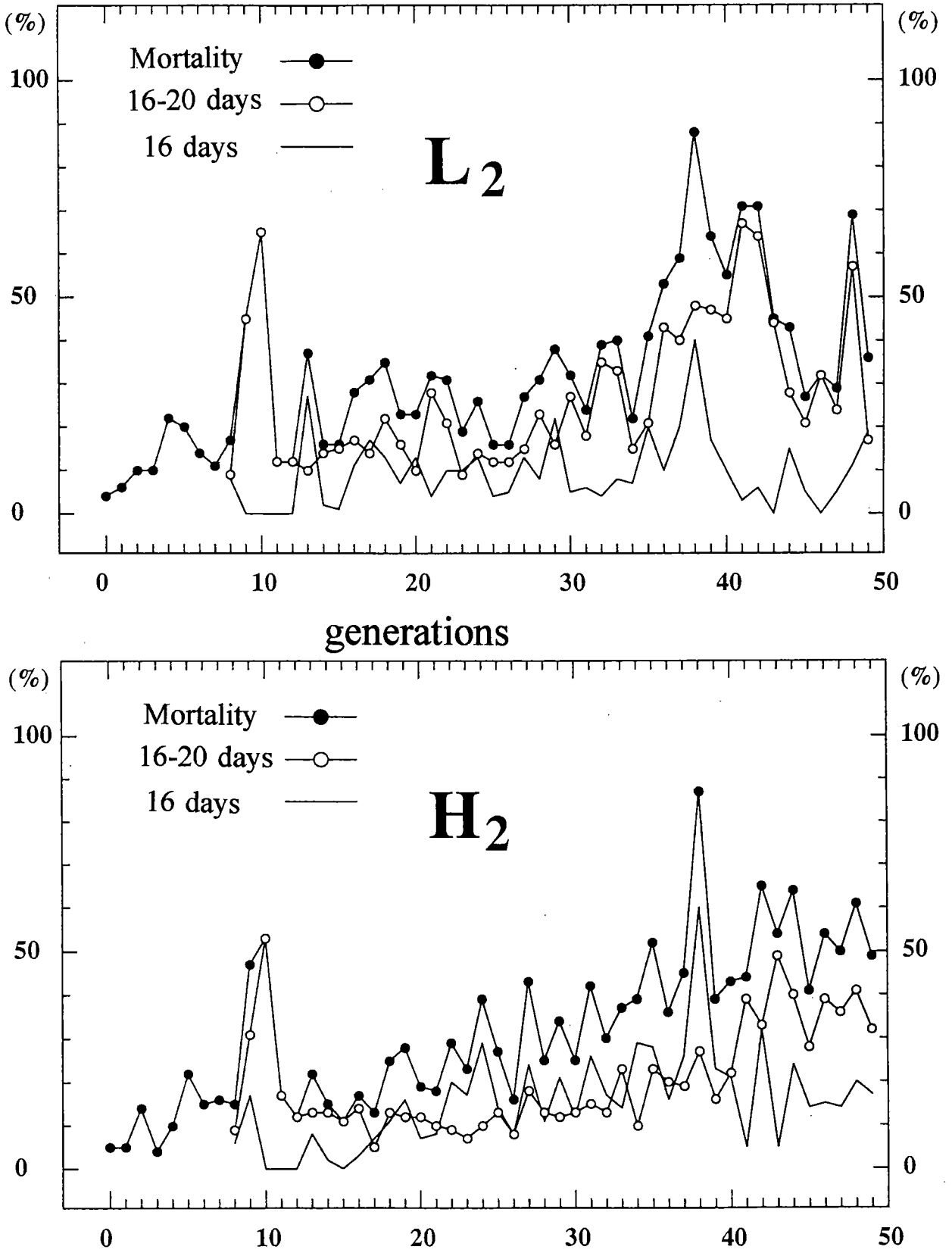

Fig.2. Changes of Embryonic Death Rate in H_2 and L_2 Quails

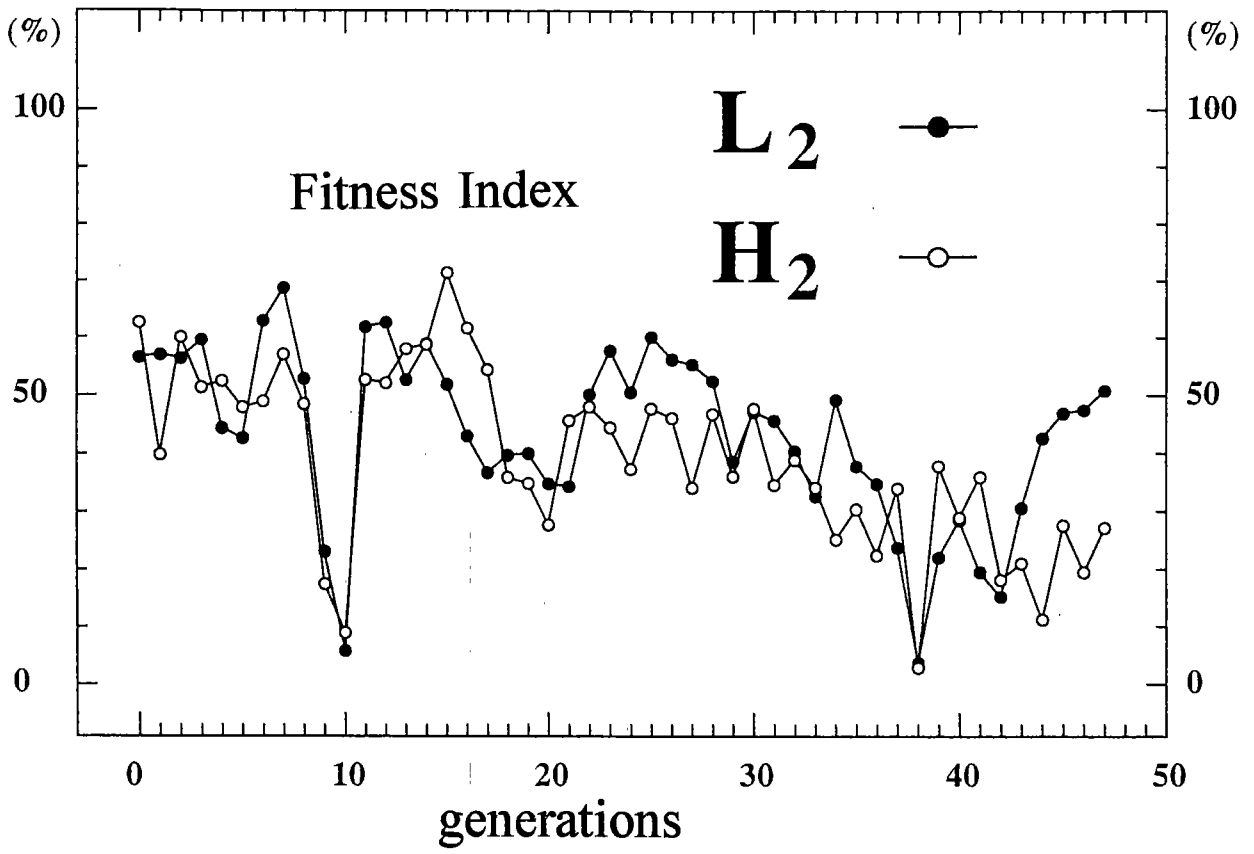

Fig.3. Changes of Fitness Index in H_2 and L_2 Quails