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Preface

Welcome to the proceedings of the 3rd International Symposium on Liberal Arts and General Education which took place at Clock Tower Centennial Hall in Kyoto University, on November 22, 2012. This was the third International Symposium on Liberal Arts and General Education sponsored by Kyoto University in cooperation with OSAKA GAS CO., LTD, The Scientific Education Exchange, and IBM Corp.

With more and more high school students being able to pursue their studies in universities, reform and enhancement of higher education have become urgent business since the quality of the graduates need to be guaranteed. Many Japanese institutes of higher education are adopting the American program, and it is often reported that this system works well. As for Kyoto University, we have tried to adopt this program for two years. We expect that our symposium would motivate the students to learn what they want so that they would become sophisticated and acquire an independent global view through this symposium.

This year, we had 92 paper submissions from undergraduate students belonging to various faculties in various types of research area. Each of the submitted papers was reviewed by graduate students. Finally we accepted 12 papers. In order to motivate students to carry out research works, the following awards are prepared: Outstanding Presentation Award, Suzuran Award, and EINSTEIN Award.

Last but not least, we would like to extend our deepest appreciation to our sponsor, corporate partners, and all the members of the Organizing Committee, especially the Symposium Chair, Miki Kioka, the Advisory Committee, Koji Koyamada and Naohisa Sakamoto. Without their invaluable contributions, this event would not have been possible.

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The Small Scale of Japanese B to C Electronic Commerce: A Proposal for 0-yen Shop

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Abstract: Japanese business to consumer (B to C) electronic commerce (EC) has been growing, and a variety of business styles have emerged. Several studies have revealed the structure of B to C EC, but we know of no study on the quantitative evaluation of B to C EC retailers and traditional retailers. The results of a comparison of the number of businesses showed that B to C EC businesses are relatively small scale. Based on this finding and previous studies, an original B to C EC plan, 0-yen shop, is developed and financially evaluated.

Keywords: B to C e-commerce, online shopping

1. Introduction

Innovations in information technology have had a huge impact on the design and management of marketing channels (Rosenbloom 1999 [1]). Online shopping is currently a major means of purchasing. According to the Japanese Ministry of Economy, Trade and Industry (2010) [2], the market scale of Japanese business to consumer (B to C) electronic commerce (EC) in 2008 was more than six trillion yen and is growing continuously.

Previous research has presented the structure of EC (Turban, Lee, King, and Chung 2000 [3]). By using the Internet, manufacturers can sell directly to customers and can provide customer support online. Thus, the traditional intermediaries are eliminated. This phenomenon is called disintermediation. However, new electronic intermediaries, such as e-malls and product selection agents, are also emerging. The occurrence of this new breed of electronic intermediaries is called reintermediation. One example of reintermediation is a price comparison web site. Profits for the 2012 fiscal year for Kakaku.com [4], a Japanese representative price comparison web site, reached eighteen billion yen, which was eighteen percent higher than the previous year.

Despite these positive data on B to C EC, the Japan direct marketing association (2000) [5] and Takahashi (2011) [6] suggest that few companies have succeeded in Japanese B to C EC. However, we know of no study comparing the scale of Japanese traditional retailing companies and B to C EC companies. I predict that the ratio of the number of small-scale Japanese B to C EC business establishments is higher than the number of physical retailing business establishments. In this study, I compare data on the number of business establishments by sales volume.

The Japanese Ministry of Economy, Trade and Industry (2010) [2] and Takahashi (2011) [6] expect that new-style EC businesses that use mobile phone online shopping will emerge in the next few years because of the rapid spread of smartphones. In this study, I develop a concrete B to C EC plan for mobile phone online shopping and estimate it using financial analysis.

2. Methods and Materials

2.1 Survey on the number of business places

A survey on B to C EC by the Japanese Ministry of Economy, Trade and Industry (2010) [2] has revealed a relationship between the number of the places of business and amount of proceeds. Based on data from the Japanese national tax agency (2010) [7], I developed a graph of the relationship between the number of physical retail business sites and the amount of proceeds, and subsequently compared the businesses.

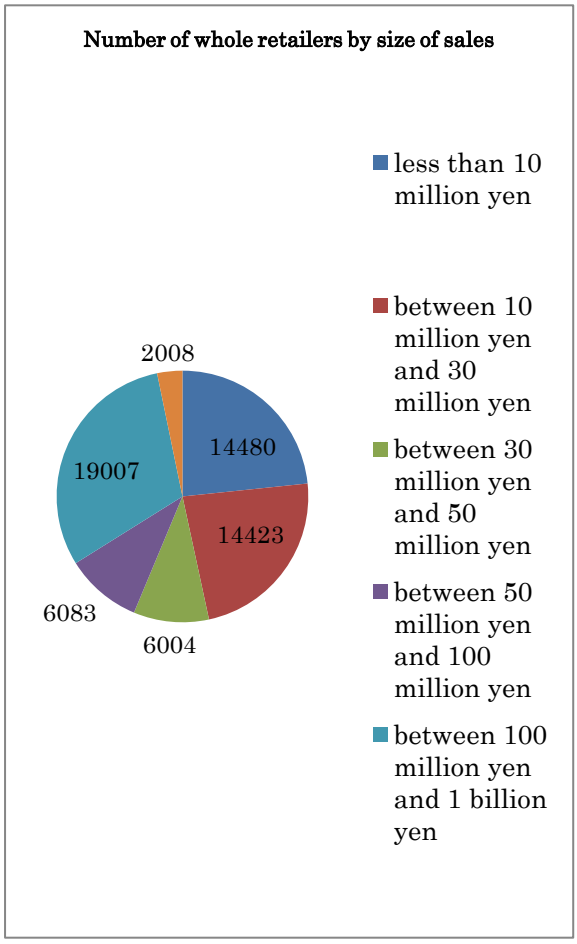
2.2 A proposal for a B to C EC plan

Applying the research results and suggestions of previous studies, I developed a B to C EC plan that uses mobile phone online shopping. The costs and revenue are estimated with reference to the "Rakuten guidebook for opening up a store" [8], TENPOlabo LTD (2012) [9], and synchro-food [10]. Then, the efficiency of the plan was evaluated by financial analysis.

3. Results and discussion

3.1 Survey on the number of business sites

A survey on the number of business sites revealed significant differences between B to C EC and whole retailers. Sixty-four percent of B to C EC companies sell less than ten million yen a year, twenty-three percent of whole retailing companies sell less than ten million yen a year (Fig. 1). This result suggests that each B to C EC business is relatively small despite its market scale. Accessibility may be a main reason for this result. For example, a customer often chooses to enter the nearest convenience store; however, in EC, all of the distances between customers and shops are the same. Moreover, there are many online shopping web sites, and customers tend to choose famous web sites for online shopping. Thus, a successful marketing strategy for B to C EC should follow a highly specific method.



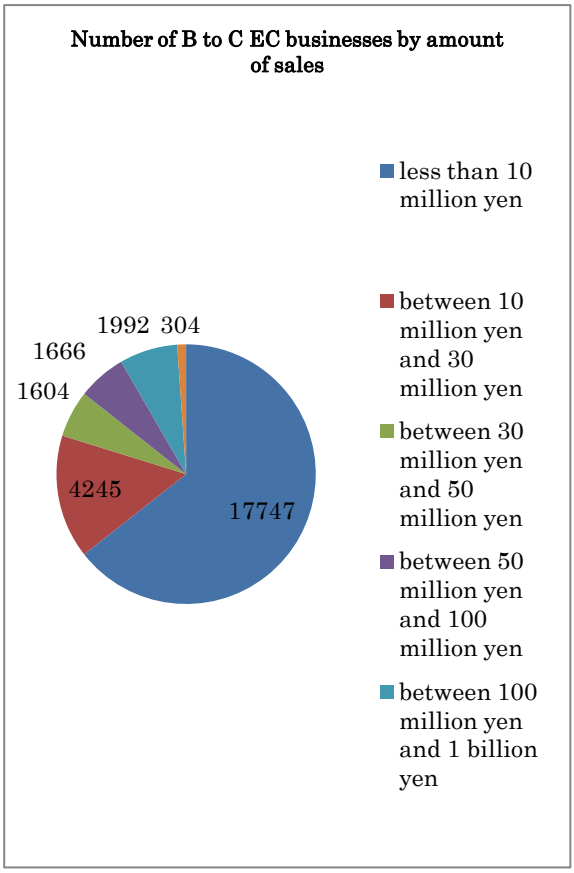


Figure 1. The number of business places by amount of sales.

3.2 A proposal for a B to C EC plan

It is important to offer high-value online shopping. The use of mobile phones for online shopping has been closely studied (Takahashi 2011; Ministry of Economy, Trade and Industry 2010). Two advantages are noted for mobile phone-based online shopping: accessibility and interactivity. Mobile phones enable people to communicate easily, thereby expanding the possibilities for online shopping businesses. In addition, smart phones have many functions, such as e-mail, memos, and social networking services. The interaction between these functions and mobile phone online shopping may create a novel form of B to C EC.

With this in mind, a new style of a B to C EC plan is developed.

The 0-yen shop: the 0-yen shop is a physical store that is offered by an internet broker. The broker is an e-mall type enterprise, such as Rakuten, the most famous Japanese e-mall, or Kakaku.com. It earns revenue from the store opening fees of contract firms. The 0-yen shop performs as a high-quality catalog and uses a specific man-

agement strategy to beat its major competitors. The concrete content of the 0-yen shop is below.

All of the items of the 0-yen shop are free approval samples, and the shop includes an eat-in corner. In fact, items are restricted from being taken from the shop. The shop offers packaged food products, health food products and cosmetic products for free as well as a variety of journals that can be read for free. The interior furniture, including tables and shelves, is also sample products. Some accessories and home appliances are displayed in the shop.

To enter and enjoy the shop, people must install the broker-developed application on their smart phone. The application has three functions: an entrance license, an e-mall browser, and a customer review writer. When customers enter the shop, "shop coins", which are original virtual money for the 0-yen shop, are consumed in proportion to time. Shop coins accumulate at consistent amounts every month, and customers can earn shop coins by purchasing items via the e-mall browser or by writing item reviews. Entrance to the shop is a motivation to purchase items. Registered companies pay the broker store opening fees depending on the size of the online shopping sale and the number of personal item reviews.

The additional cost of starting the 0-yen shop is estimated below.

The total initial cost of the 0-yen shop is estimated to be 16.00 million yen per shop, which is derived from the estimation example of TENPOlabo LTD (2012). The location is the city center. The total administrative costs of the 0-yen shop are tentatively estimated at 1.10 million yen per month per shop. It is assumed that there are no prime costs for shop items.

In this situation, break-even revenue is estimated at 1.37 million yen per month when the cost is paid off in five years. In this plan, 17.2 contract companies who sell 0.5 million yen per month are needed, based on the reference data on store opening fees from Rakuten, to meet this mark. That is, monthly additional online shopping sales must be 8.61 million yen per shop.

4. Conclusion

Although Japanese B to C EC businesses have a large potential market, they are relatively small scale, and a variety of B to C EC businesses have emerged. In particular, mobile phone online shopping is expected to grow in the next several years. This paper suggests a 0-yen shop that integrates an e-mall and a physical shop. However, the estimation implies that this plan is not financially affordable for small and medium enterprises, and effective advertising and propaganda may be needed for the practical use of the 0-yen shop. Various business styles and customer behavior toward new online services require further study.

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Mismatch repair protects against γ-rays in *Caenorhabditis elegans*

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Abstract: It is known that the non-homologous end-joining (NHEJ) and homologous recombination (HR) pathways are used to repair DNA damage due to radiation, ultraviolet (UV) light, or oxidation. Although mismatch repair (MMR) plays an important role in the HR pathway, no experiment has demonstrated the relationship between MMR and protection against radiation-induced damage. MLH1, MSH2, and EXO1 are three MMR-related factors [1] [2]. In this study, each of these factors was knocked down in *Caenorhabditis elegans* (*C. elegans*) using RNA interference (RNAi). The RNAi-treated *C. elegans* were irradiated with γ-rays, and then their life spans were measured. We hypothesized that the life span of irradiated *C. elegans* would be shorter than that of unirradiated *C. elegans* because of the lack of a MMR-related factor that is involved in the repair of DNA damage due to radiation. Thus, the DNA damage due to the γ-rays would remain and would have a negative effect on the life span. However, our experiments showed that there was no change in the life span of *mlh1*(RNAi) *C. elegans* or *msh2*(RNAi) *C. elegans* and that the life span of *exo1*(RNAi) *C. elegans* was extended.

Key Words: mismatch repair, γ-rays, life span, *Caenorhabditis elegans*

1. Introduction

Organisms maintain and transmit genetic information through DNA. However, radiation, UV light, and oxidation by reactive oxygen species damage DNA. It is known that such DNA damage is repaired by non-homologous end-joining (NHEJ) and homologous recombination (HR). NHEJ is essentially a mechanism that connects the DNA ends that are produced by double-strand breaks (DSBs). NHEJ can occur during any stage of the cell cycle, but NHEJ is most frequent during the stage before DNA replication, during which HR cannot proceed. Unlike NHEJ, HR requires the sister chromosome as a template to repair cleavage sites, including DSBs. Damaged DNA can be repaired by HR only when the newly produced sister chromatid that has the same sequence is available. Because of this requirement, HR is used to repair DNA predominantly during or after DNA replication (refer to Appendix Figure 7-1). NHEJ tends to produce numerous mutations. Some biologists consider NHEJ to be a temporary repair mechanism that repairs DNA damage prior to DNA replication; later, HR may be used to make the final repair to the damaged site because HR is more accurate [unpublished]. Therefore, whenever DNA damage occurs, HR is the final repair mechanism used.

Although it is known that MMR plays an important role in HR, there has been no study that demonstrates the relationship between MMR and protection against radiation-induced damage. MMR is the pathway that repairs incorrect insertions, deletions, or mismatches of nucleobases that occur during HR, DNA replication, and genetic recombination. If DNA damage sites due to radiation are not removed, they can cause cell death. Therefore, there should be many radiation defense mechanisms. In addition, previous studies have shown that MMR is related to cancer and aging. Furthermore, we hypothesize that the inaccuracy of MMR may contribute to evolution by giving rise to biodiversity. Because of these facts and ideas, we are very interested in MMR, and we are trying to understand MMR-related factors and to elucidate the MMR mechanism in detail.

In this experiment, we attempted to determine whether there was any difference in life span due to the knockdown of MMR-related factors when DSBs or other types of DNA damage occurred. Knockdown means suppressing function. DSBs are expected to undergo final repair by HR, and HR requires MMR. Consequently, MMR is required to repair DSBs. We focused on γ-rays as a cause of DNA damage, specifically DSBs, to research the relationship between MMR and radiation defense because γ-rays have been used to cause DSBs in many previous studies.

Thomas E *et al.* revealed that when wild-type *C. elegans* was irradiated with 1000 grays (Gy) of γ-rays, the life span was similar to or longer than that of un-

irradiated *C. elegans* and that doses above 1000 Gy were required to reduce the mean life span of *C. elegans* [3]. However, in this experiment, 100 Gy of γ-rays was employed because this dose was considered the maximum dose from our radiation device that would not cause other stresses in *C. elegans*. In addition, it has been demonstrated that even if each MMR-related factor (MLH1, MSH2, or EXO1) is knocked down, the life span of *C. elegans* is still similar to that of the wild type [unpublished].

In this study, we performed an experiment to test the theory that if each of the MMR-related factors (MLH1, MSH2 or EXO1) is knocked down by RNAi in *C. elegans* and then the *C. elegans* are irradiated with γ-rays, the life span will be shorter than that of unirradiated *C. elegans* (refer to Appendix Figure 7-2).

RNAi is a phenomenon that inhibits the expression of a gene by means of the introduction of a double-stranded RNA that has a sequence homologous to that of the target gene. RNAi was discovered in *C. elegans*. In this experiment, *C. elegans* was cultured with transformed *Escherichia coli* (*E. coli*) as a food source. The *E. coli* were also used to induce RNAi. Transformed *E. coli* were generated that contained plasmids to individually suppress the expression of each gene (*mlh-1*, *msh-2*, and *exo-1*) in *C. elegans* indirectly using double-strand RNAs. When the *C. elegans* consumed these bacteria, the expression of the homologous *C. elegans* gene (*mlh-1*, *msh-2*, or *exo-1*) was suppressed. That is, when the RNAs for RNAi were generated in *E. coli* and then the *C. elegans* ate those *E. coli*, the targeted factor (MLH1, MSH2, or EXO1) in *C. elegans* was knocked down.

2. Materials and Methods

C. elegans was used in this experiment. This organism is a transparent nematode of approximately 2 mm in length and is a model organism because it has many experimental merits.

When it is starved at the proper growth stage, wild-type *C. elegans* changes into a dauer that stops growing completely at a certain stage. However, if food, e.g., *E. coli*, is provided, the *C. elegans* dauer resumes growth. In this experiment, the *C. elegans* mutant fem-3(q20), which cannot produce any eggs at 25°C, was used so that the dead *C. elegans* could be counted accurately (no new *C. elegans* could be born). *C. elegans* was cultured on plates with *E. coli*.

First, *C. elegans* (fem-3(q20)), which had been stored at 16°C as a dauer, was passaged onto a plate seeded with *E. coli* to allow the *C. elegans* to mature and produce eggs at 2°C. *C. elegans* containing eggs were dissolved to collect the eggs (the eggs were not dissolved because of their eggshells), and then the eggs were allowed to hatch at 20°C. *E. coli* transformed with plasmids encoding homologous sequences were used as food for the nematodes so that RNAi occurred to knock down the target factor in *C. elegans*. Transformation means to change the genetic makeup of *E. coli* by introducing a plasmid for a specific purpose. Hatched *C. elegans* were fed on transformed *E. coli*. Finally, the *C. elegans* were irradiated with 100 Gy of γ-rays, and the number of dead *C. elegans* was counted every 2 days to measure the life span.

(The protocol and a flowchart of the methods are shown in Appendix 7-3)

3. Results

According to t-tests performed using statistical analysis software [4], the life span of the irradiated control was similar to that of the unirradiated control because the p-value was 0.1419, which is greater than 0.05 (Figure 1). The term "control" means *C. elegans* in which no genes were knocked down. The p-value is a statistical indicator. If it is less than 0.05, the two things being compared can be considered different. However, if the p-value is more than 0.05, these two things can be regarded as almost the same. Therefore, it can be concluded that γ-rays had no effect on the life span of the control nematodes. Similarly, irradiated *mlh1* (RNAi) and *msh2* (RNAi) *C. elegans* had life spans similar to those of the unirradiated *mlh1* (RNAi) and *msh2* (RNAi) *C. elegans* because the p-values were 0.4452 and 0.2351, respectively, each of which is greater than 0.05. *mlh1* (RNAi) and *msh2* (RNAi) refer to *C. elegans* whose MLH1 or MSH2 gene was knocked down, respectively. Therefore, even if MLH1 or MSH2 was knocked down and DNA damage by γ-rays would not be repaired normally, life span did not shortened. Unexpectedly, the life span of the irradiated *exo1* (RNAi) *C. elegans* can be considered longer than that of the unirradiated *exo1* (RNAi) *C. elegans* because the p-value was 0.0388, which is less than 0.05. *exo1* (RNAi) refers to *C. elegans* whose EXO1 gene was knocked down. This result also indicates that even if EXO1 is knocked down and the DNA damage caused by γ-rays cannot be repaired normally, the life span is not shortened. Although there were some small differences, as shown in Figure 1, no significant differences were found.

In this experiment, approximately 30 individuals of each of the four types of irradiated worms and unirradiated worms were used. Thus, this experiment used a total of approximately 240 animals. This small sample size may have prevented some of these comparisons from being statistically significant.

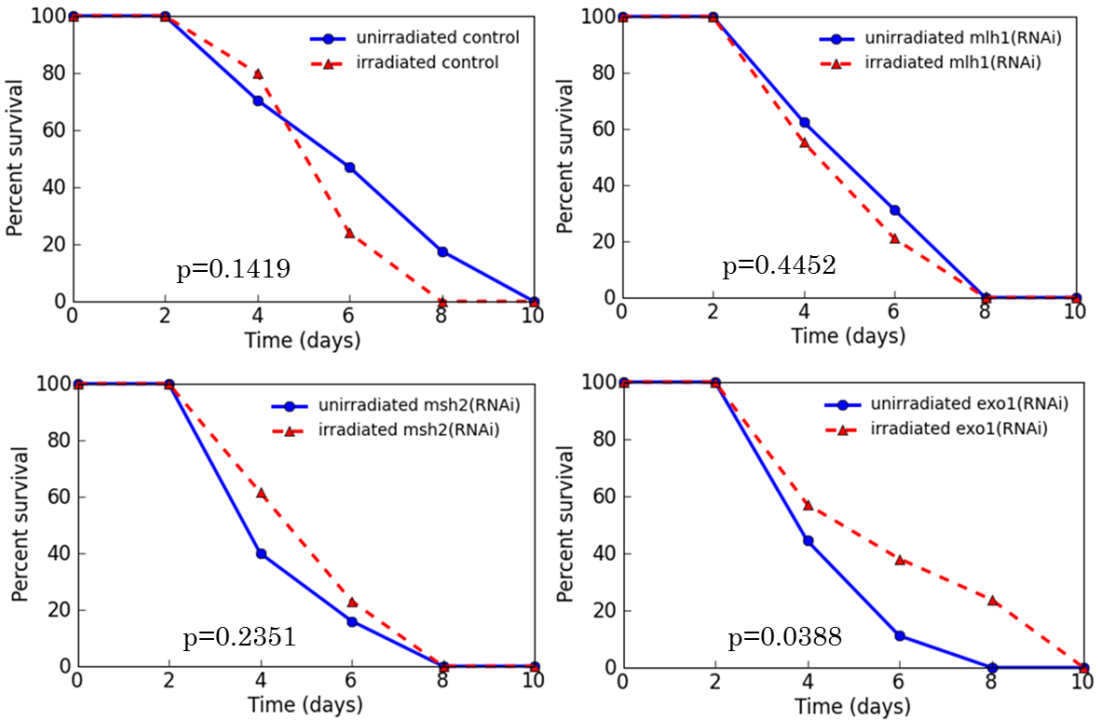

Figure 1. Percent survival after exposure to γ-rays

Control *C. elegans* were not subjected to RNAi. *mlh1* (RNAi), *msh2* (RNAi), and *exo1* (RNAi) refer to *C. elegans* whose MLH1, MSH2, and EXO1 genes were knocked down, respectively. "p" indicates the significance probability, and "Time (days)" represents the number of days after irradiation.

4. Discussion

According to a previous study, even if each of these MMR-related factors (MLH1, MSH2, and EXO1) is knocked down in *C. elegans*, the life span of *C. elegans* is similar to that of wild-type *C. elegans* [unpublished]. In this study, each of these factors was knocked down, and then the *C. elegans* were irradiated with γ-rays. For the *mlh1* (RNAi) and *msh2* (RNAi) *C. elegans*, there was no difference in the life span between the irradiated and unirradiated groups. However, for the *exo1* (RNAi) *C. elegans*, the life span of the irradiated *C. elegans* was longer than that of the unirradiated nematodes.

These results suggest that there is another MMR mechanism that does not involve MLH1 or MSH2, that MLH1 and MSH2 play minor roles in MMR, or that there is a powerful backup mechanism that does not involve EXO1 that results in the extension of the life span. It can also be suggested that *C. elegans* can acquire resistance to γ-rays.

Some limitations of this study must be acknowledged. First, the number of dead *C. elegans* was counted only every 2 days, but the *C. elegans* died sooner than expected. Therefore, the number should have been counted every day. In addition, each individual affected the survival rate because of the small sample size. For this reason, in the future, we should use a larger sample size. Furthermore, it will be easier to discuss the results if we focus on only one factor in each experiment. In addition, we should assess the efficiency of the RNAi by RT-PCR or western blot analysis, and we should use several different doses of γ-rays to damage the DNA.

5. Conclusion

In *C. elegans*, even if MLH1 or MSH2, both MMR-related factors, was knocked down by RNAi and then *C. elegans* were irradiated with γ-rays, there was no significant difference between the life span of the irradiated and unirradiated groups. However, when EXO1, another MMR-related factor, was knocked down and then the *C. elegans* were irradiated, the life span was extended relative to that of the unirradiated nematodes.

Therefore, the hypothesis that the life span would decrease if each of three MMR-related factors in *C.*

elegans (MLH1, MSH2, or EXO1) were knocked down and then the *C. elegans* were irradiated with γ-rays was not supported. However, significant results were obtained. Even when a MMR-related factor was knocked down, γ-ray-induced damage did not shorten the *C. elegans* life span. In other words, MMR protects against γ-rays.

In the future, we need to repeat this experiment and pay greater attention to the points mentioned in the discussion to confirm the validity of these results. We also need to answer the following questions: Why was the life span of irradiated *C. elegans* in which MLH1 or MSH2 was knocked down not shorter than that of unirradiated *C. elegans*? And why was the life span of irradiated *C. elegans* in which EXO1 was knocked down extended relative to that of unirradiated *C. elegans*?

The elucidation of the MMR mechanism will improve our understanding of cancer and aging because MMR is closely related to these processes. In addition, if this mechanism is understood, we will also be able to understand the evolutionary process that produces biodiversity because the inaccuracy of MMR can be regarded as a cause of mutations that remain in DNA.

6. References

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7. Appendix

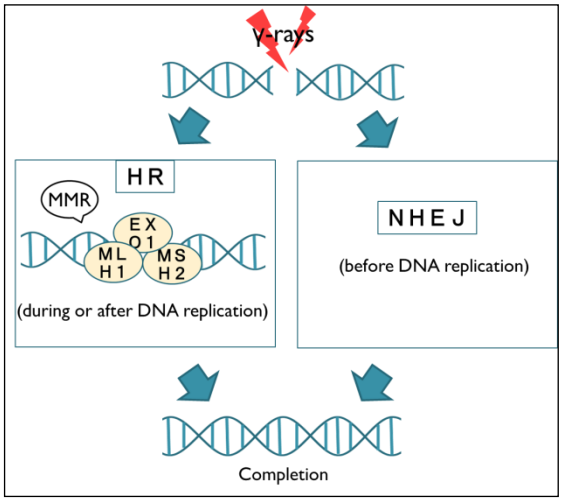

Figure 7-1. A schematic representation of DNA damage repair.

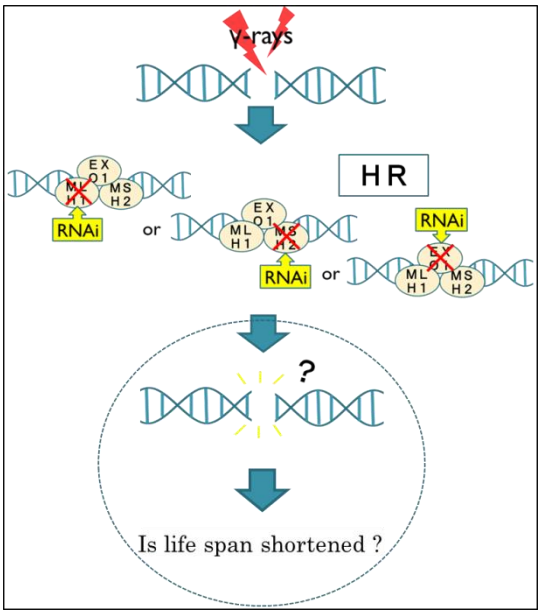

Figure 7-2. An image describing what was examined in this experiment.

7-3. Protocol.

The methods below are followed by a flowchart of the methods.

2-1. Passage

(1). On a clean bench, remove a portion of the agar from the stock *C. elegans* (fem-3(q20)) plate, and then put it on a new NGM plate (0.3% NaCl, 0.25% polypeptone, 0.002% cholesterol, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM potassium phosphate (pH 6.0), 1.8% agar).

(2). Wrap a piece of parafilm around the plate, and then culture it in the dark for 2-3 days.

2-2. Recovery of Eggs

(1). Pour 1 ml of distilled water (D1) onto the passaged *C. elegans* plate, and collect *C. elegans* in a 1.5 ml tube.

(2). Centrifuge this tube.

(3). Remove the supernatant, leaving 100 µl of the contents.

(4). Add 600 µl of D1, 200 µl of 6% hypochlorous acid, and 100 µl of 5 M NaOH.

(5). Let the tube stand at room temperature for 5-10 minutes.

(6). Vortex and centrifuge the tube, and then remove the supernatant, leaving 100 µl of its contents.

(7). Pour 1 ml of S-basal medium (100 mM NaCl, 50 mM potassium phosphate (pH 6.0)) into the tube, vortex it, and centrifuge it.

(8). Repeat (7) three times.

(9). Culture the contents of the tube at 20°C for 2-3 days to hatch the eggs.

2-3. Transformation

(1). Add 1 µl each of pPD 129.36 -msh-2, pPD 129.36 -mlh-1, pPD 129.36 - exo-1, or pPD 129.36 to 10 µl of BL-21 competent cells (*E. coli*, $CaCl_2$, glycerol).

(2). Place the tube on ice for 30 minutes.

(3). Heat shock the *E. coli* at 42°C for 1 minute.

(4). After leaving the tube on ice for 2 minutes, add 100 µl of liquid LB (Luria-Bertani) (1.0% polypeptone, 0.5% yeast extract, 0.5% NaCl, NaOH (pH 7.2)), spread the contents of the tube onto an LB plate (2.0% agar powder, 1.0% polypeptone, 0.5% yeast extract, 0.5% NaCl, NaOH (pH 7.2)), and culture the plate at 37°C for 14-15 hours.

(5). Store the plate at 4°C.

2-4. Induction

(1). Add 5 µl of Cb and one colony of *E. coli* stored at 4°C to 5 ml of liquid LB.

(2). Culture the *E. coli* at 37°C for 17 hours with shaking.

(3). Add 50 µl of the culture from (2) to 5 ml of fresh liquid LB to dilute the culture 100 times.

(4). Add 5 µl of Cb.

(5). Culture the sample from (4) at 37°C for 2 hours with shaking.

(6). Add 18 µl of 100 mM IPTG.

(7). Culture the *E. coli* at 37°C for 4 hours with shaking.

(8). Add 18 µl of 100 mM IPTG.
(9). Take 500 µl of the culture form (8), spread it onto a NGM plate, and dry the plate.

2-5. RNAi
(1). Transfer hatched *C. elegans* onto the plate prepared as described above in session 2-4.
(2). Incubate the plate at 25°C in the dark.

2-6. Irradiation with γ-rays
(1). Spread 1 ml of M9 buffer onto an RNAi plate and collect the *C. elegans* in a 1.5 ml tube.
(2). After tapping the tube and pipetting the contents, removed 450 µl from the tube, and put it into a new 1.5 ml tube.
(3). Mark the tubes that will be irradiated.
(4). Take all tubes into the room with the γ-rays radiation device.
(5). Irradiate the marked tubes with approximately 100 Gy (0.83 Gy/min×120 min) of γ-rays.
(6). On a clean bench, drop 70 µl of transformed *E. coli* as food onto the center of a NGM plate and dry it.
(7). Place a few drops of irradiated *C. elegans* of (5) onto the plate made in step (6) so that each plate contains approximately 15 individuals.
(8). After drying the plate on a clean bench, wrap a piece of parafilm around the plate, and store it in the dark at 25°C.

2-7. Measurement of the Life Span
(1). Count the number of dead *C. elegans* every 2 days until the entire population is died.
(2). When the *C. elegans* are not being observed, they should be stored in the dark at 25°C.

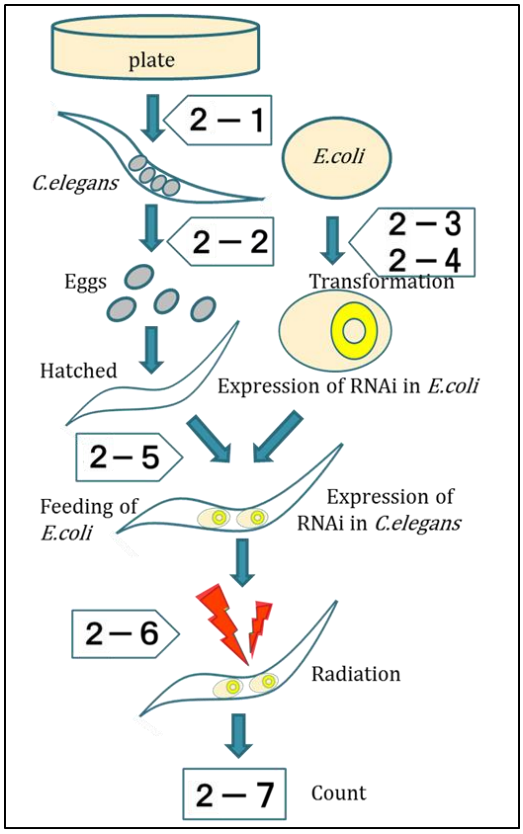

Figure 7-3. A flowchart of the methods.

First, *C. elegans* (fem-3(q20)) that has been stored at 16°C as a dauer is passaged and grown at 20°C to allow the nematodes to mature and produce eggs (2-1). *C. elegans* containing eggs are dissolved to collect the eggs (the eggs are not dissolved), and then the eggs are placed at 20°C for hatching (2-2). *E. coli* containing plasmids that include homologous sequences are prepared for use as food so that RNAi occurs in *C. elegans* to knock down the targeted factors (2-3, 2-4). Hatched *C. elegans* eat the *E. coli* (2-5). Finally, *C. elegans* is irradiated with 100 Gy of γ-rays (2-6), and the number of dead *C. elegans* is counted every 2 days (2-7).

Is *The Izeki Takako Diary* a Work of Diary Literature?

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Abstract: *The Izeki Takako Diary* (hereafter referred to as *the Diary*), written in the Edo era, has been treated chiefly as a historical document. However, previous studies have suggested that *the Diary* should be classified as diary literature. Generally speaking, however, *the Diary* has not been accepted as a work of literature. In this study, we considered the requirements for a diary to be classified as 'diary literature' and investigated whether *the Diary* satisfies those requirements. We conclude that *the Izeki Takako Diary* should be regarded as diary literature. Our findings may prompt further studies of diary literature in the Edo era and clarify the division between a diary and diary literature, which will be beneficial for both literature scholars and historians.

Key Words: *the Izeki Takako Diary*, diary literature

1. Introduction

Izeki Takako (1783---1844), who lived at the latter end of the Edo era, was a *samurai*'s wife. She kept a detailed diary from *tempō* 11 (1840) to *tempō* 15 (1844). Today the diary is known as *the Izeki Takako Diary* (*The Diary* hereafter), and it includes records kept over a period of 898 days.

The Diary has been treated mainly as a historical document because it accurately describes many historical facts. However, in 2004, Akio Fukasawa suggested in his book *Izeki Takako no kenkyu*[1] that *the Izeki Takako Diary* should be evaluated as diary literature.

Nevertheless, *the Diary* is rarely seen as diary literature. Indeed, few books on the history of Japanese literature mention *the Diary*. Generally speaking, it is viewed chiefly as a 'diary', not as 'diary literature'.

One of the major reasons for the classification of this work as a diary may be that most studies of diary literature are related to works written in the Heian era. Thus, the field of diary literature tends to mainly include Heian letters, and the general definition of 'diary literature' is applied only to the texts of the Heian era. For this reason, *the Izeki Takako Diary*, which was written in the Edo era, is not thought of as a work of literature.

Furthermore, Akio Fukasawa, who attempted to extend the definition of 'diary literature' so that it could be applied to the text written in the Edo era, applied ambiguous criteria. He considered *the Diary* to be diary literature because the writing is clear and because its contents are interesting and exciting. However, this judgment is somewhat subjective, and Fukasawa's opinion is not sufficient.

In this study, the question of whether *the Izeki Takako Diary* can be classified as diary literature is investigated by applying the definition of 'diary literature' to the works of the Edo era. The distinction between a 'diary' and 'diary literature' is important because the former should be studied by historians, while the latter should be studied by literature scholars. Thus, this study may be beneficial for both historians and literature scholars in that the subjects of both fields are clarified.

2. The definition of 'diary literature'

First, we consider what aspects are necessary to classify a diary as 'diary literature'.

Nihon kotembungaku daijiten[2] provides one definition of 'diary literature':

Kōgi no nikkibungaku toha, tokutei no jitai ya gyōji, aruiha nichijōtekina jitsumu, kōsi no seikatsu, kembun nado wo kirokusuru nikki no naka de, tokuni hissha no shutaitekina kōi ya jinseikan ga tsuyoku arawasa re, naiseiteki, kandōtekina naiyō wo mot ta mono wo iu.

[Diary literature, in a broad sense, is a diary that greatly expresses the author's subjective actions or view of life and includes introspective and impressive contents, while a diary is simply a

record of specific matters, events, everyday work, official or private lives and experiences, and so on.]

In summary, the authors' subjective emotions or thoughts must be strongly expressed in 'diary literature', whereas a 'diary' is merely a document that records what has happened. Actually, famous works of diary literature, such as *the Tosa Diary* and *the Sarashina Diary*, include the authors' feelings. Thus, this feature is one of the requirements for a diary to be classified as a work of literature.

Next, to further define the characteristics of diary literature, the letters and characters included in the work should be carefully considered. By identifying the common features of the diary literature from the Heian era, it is evident that these works were written mainly in *kana* characters. This contrasts with the fact that public records and documents were written in Chinese characters, and they took the form of Chinese writing, or *kambun*. Therefore, whether a text is written in *kana* characters or in the form of *kambun* may represent a distinction between diary literature and diaries as historical records.

In this regard, *the Tosa Diary*, written by Ki no Tsurayuki, gives us an interesting perspective. Although it was the custom for men to write documents in Chinese characters at that time, Ki no Tsurayuki wrote his diary in *kana* characters from the perspective of a woman. *The Tosa Diary* may be considered a work of literature because Tsurayuki wrote it in *kana* instead of *kambun*. Therefore, it seems to be an important distinction that diary literature is not written in Chinese characters.

This feature represents the second characteristic of diary literature. Here, 'not being written in the form of Chinese writing' weighs more heavily than being written in *kana* characters, in that diary literature is distinct from diaries that provide accounts of facts. Therefore, a requirement of diary literature is that it cannot be written in Chinese characters.

In addition, we can define one more characteristic of diary literature based on the common features shared by the diary literature of the Heian era: The authors wrote these works at an old age, recalling how they had led their lives. Ken Akiyama discusses this matter in *Nikkibungaku jiten*[3]:

Nikkibungaku no sekai ha, sono sekai no naka wo keikashi ta jikan ni taiōsuru tokoro no, sakusha no jitsujinsei no kikan yori ato ni, kaisō to shi te tsumugidasa re ta mono de aru.

[The world described in diary literature was created from the author's memories of times that had passed.]

This principle is difficult to apply to the text written in the Edo era, though it is applicable to works from the Heian or Kamakura era because works that took the form of recollection became fewer and fewer after medieval times. Instead, diaries that were kept every few days began to appear. Because we aim to provide a definition of 'diary literature' that can applied to the text in the Edo era, the principle mentioned above needs to be modified somewhat.

Thus, we should consider what effect the act of recollection produces. When the author looks back on his or her life and attempts to write about it, it is impossible to capture all of the events that happened. This means that authors are forced to choose what to write and what to leave unwritten. Therefore, in a broad sense, writing diaries by recalling various things is equivalent to selecting the contents of the work. The presence of this selection is the third characteristic of diary literature.

Collectively, 'diary literature' must fulfill the three requirements listed below.

A) The author's subjective feelings need to be expressed strongly in the work.

B) The work must not be written in Chinese characters.

C) What to write and what to leave unwritten in the work must be selected by the author.

In the next chapter, we will consider whether *the Izeki Takako Diary* meets these requirements.

3. The case of *the Izeki Takako Diary*

First, we will review whether *the Izeki Takako Diary* meets requirement B before considering whether it meets requirement A. *The Diary* was written chiefly in *kana* characters and partly in *kanji*, but it was not written in the form of Chinese writing. Therefore, requirement B is satisfied.

Second, our next concern is whether *the Diary* fulfills requirement A. To consider this question, the first two sentences of *the Diary* are cited:

Munemuneshiki koto ha ōyake ni shirusa re, hata saranu koto domo mo, yo no hito no kashikoki fude ni onogajishi shirusu beka mere ba, toritatete nanigoto kaha iha re mu. Shikare domo tsurezurenaru mono no susabi ni ha, hakanaki koto wo mo shirushi tsutsu, kokoro wo yaru yori hoka no nagusame namu naki.

[Important events are recorded by the government, and many private affairs are also written by intelligent people. That is why nothing seems to be left for me to write about in this diary. For me,

nevertheless, it is the only comfort to enjoy writing down unimportant things because I am old and bored.]

Takako declared that she would write about unimportant and personal things instead of public and official records or the various contents of documents written by the government or highly intelligent people. Therefore, her own emotions and opinions are strongly expressed in *the Izeki Takako Diary*.

For instance, she sharply criticizes Confucians in the entry written on August 22, *tempō* 12 (1841). She respected those who had studied Japanese classical literature, *kokugakusha*, who tried to exclude Chinese thought. Therefore, Takako logically found fault with Confucianism in favor of *kokugakusha*.

Furthermore, *the Diary* contains approximately 800 Japanese poems (*waka* and *chōka*) written by Takako, in which she describes her feelings. For example, the following poem describes her feelings when she saw a vase of *ume* flowers falling:

Haru shi ko ba mata koso saka me ume no hana chiri te kahera nu hito no yo zo uki

[Whereas *ume* flowers will bloom again in the next spring, humans cannot return once they have passed away. How trying this world is!]

Thus, Takako strongly expresses her beliefs and feelings in *the Diary*. Therefore, we can conclude that *the Diary* fulfills requirement A.

Finally, we must consider whether *the Diary* meets requirement C. First, we must consider how Takako led her life. Before she married Chikaoki Izeki at the age of approximately 30, she had experienced a divorce from her first husband, Gen'emon Matsunami. (Fukasawa, 2004 [1]) For her, the failure of the first marriage must have been a crucial and distressing event. Perhaps because of the shock, she did not write about her marriage to and her divorce from Gen'emon at all in *the Diary*, although it is noteworthy that she did write about her childhood memories.

In *the Izeki Takako Diary*, she honestly expressed her affection for her family and also recalled her past experiences, and yet she never wrote about her first marriage. This suggests that she deliberately avoided writing about that topic. She may have wanted to forget the shocking divorce by leaving the event unrecorded and unwritten. Thus, it is clear that she selected the contents written in *the Diary* subjectively, thus satisfying requirement C.

4. Conclusion

In conclusion, we conclude that *the Izeki Takako Diary* is a work of 'diary literature' as it satisfies all the requirements mentioned in chapter 2.

Generally speaking, diary literatures written in the Heian era have been well researched by a great number of scholars. However, there have been far fewer studies of diary literature in the Edo era. Thus we hope that additional research on diary literatures in the Edo era will be conducted as a result of this study.

Furthermore, this study may improve our understanding of the distinction between a 'diary' and a work of 'diary literature'. To separate these two types of work is of vital importance to clarify the subjects of history and literature. In that sense, this research may contribute to both fields.

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The Trigger for Feeding in *Aurelia aurita*

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Abstract: This study investigated the trigger for feeding in *Aurelia aurita* polyps and the relationship between feeding and hunger in the polyps. A needle was used to stimulate the polyps, and their reactions were observed. Fasted polyps frequently responded to non-food items. The ability of polyps to distinguish food from non-food items physically or chemically was investigated by exposing polyps to needles, to glass beads, and to solutions of fermentum, peptone, and glucose. The polyps responded to the needles more often than to the glass beads. They responded to fermentum and peptone but not to glucose. Glass beads resemble Artemia more than do needles. These findings show that the polyps use chemical cues to distinguish food from non-food items. The polyps responded to fermentum more strongly than to peptone. This result indicated that polyps use many substances as key factors. This result was not consistent with results from a study of another type of polyp (*Cordylophora*), which distinguishes food based on only one type of amino acid. This finding suggests that the feeding tendencies studied differ among jellyfish species and require further study with each type of amino acid.

Key Words: *Aurelia aurita* polyps, feeding, trigger, amino acid.

1. Introduction

Aurelia aurita polyps catch zooplankton in their tentacles and convey the food to their open mouths. This foraging activity of the polyps is triggered when the zooplankton touch the polyps' tentacles. However, items other than zooplankton are also present in seawater. Therefore, the polyps must be capable of distinguishing zooplankton from non-food items.

Figure.1 a polyp of *Aure-*

C. Fulton identified the trigger for foraging activity in *Cordylophora* (Fluton, 1963[1]). According to Fulton's study, proline, a type of alpha amino acid, can trigger foraging activity in *Cordylophora.* However, many animals recognize food based on cues other than amino acids (i.e., sugar and fat). Therefore, it is unclear whether polyps recognize food based only on amino acid cues. If polyps recognize food based only on amino acid cues, it is also unclear that the polyps use only one type of amino acid as a cue. It is reasonable to raise this question because approximately 20 different amino acids exist.

In this study, the trigger for the foraging activity of *Aurelia aurita* polyps was investigated and compared with the previous findings for *Cordylophora*.

In the present study, a key substance (such as proline) could not be identified. However, identifying the key substance is important for considering differences among jellyfish and the evolutionary history of jellyfish.

2. Methods

The polyps used in these experiments were cultured from 6 polyps collected in Ise Bay. These polyps were cultured according to the methods of D. B. Spangenberg (Spangenberg,Varnigonnia). The polyps were cultured in petri dishes at a temperature of 20±2℃. Every 3 days, the polyps were fed newly hatched Artemia. The polyps were cultured in artificial seawater (LIVE Sea Salt by DELPHIS/Japan).

(1) Experiment 1

-Can polyps distinguish food from non-food items?-

This experiment investigated the possible ability of polyps to distinguish Artemia from iron needles. A total of. 51 polyps were exposed to an iron needle, and 18 polyps were exposed to Artemia. The reactions of each polyp were observed.

(2)Experiment 2

-Does hunger affect the reactions of polyps?-

This experiment investigated the effects of hunger on the reaction of polyps to an iron needle. Two groups of polyps were formed. In one group, polyps were fed normally (118 polyps). In the other group, polyps were fasted for 10 days until the experiment was conducted (20 polyps). The color of the normally fed polyps was orange, derived from the Artemia, and the color of the fasted polyps was milky wneedle, and their reactions were observed.

(3) Experiment 3

-Do polyps distinguish food physically or chemically?-

This experiment investigated the possible ability of polyps to distinguish Artemia from non-food items physically or chemically. To determine whether the polyps use physical cues for this purpose, it is necessary to observe the reactions of the polyps to stimuli that are more Artemia-like than the needles used in previous experiments. For this purpose, glass beads (approximately 0.6 mm, nearly the same size as Artemia) were presented to 20 polyps, and the results of this experiment were compared with the results obtained with the needles in Experiment 2 (fasted polyps). To determine whether the polyps use chemical cues to distinguish Artemia from non-food items, three groups of 20 polyps were formed and exposed to peptone, fermentum, and glucose at concentrations of 1.0×10^{-2} g/L, 1.0×10^{-3} g/L, and 1.0×10^{-4} g/L, respectively. These three substances were dissolved in artificial seawater (LIVE Sea Salt, by DELPHYS).

The results of the observations were classified according to three categories: reacted (the tentacles constricted) and opened mouth, reacted and did not open mouth, did not react. In experiment 3, however, the occurrence of constriction could not be evaluated because the hydraulic pressure produced by the presentation of the experimental solutions caused the polyps' tentacles to move. Therefore, in the observations of the response to physical cues "reacted and did not open mouth" was recorded as "did not react" in experiment 3.

Only polyps in good condition were used in these experiments. For this reason, the number of polyps is unequal across experimental tests.

3. Results

Figure 2shows the response of polyps exposed to needles and to Artemia, All the polyps exposed to Artemia responded, and 95% of the polyps opened their mouths. However, only 40% of the polyps exposed to the needle opened their mouths.

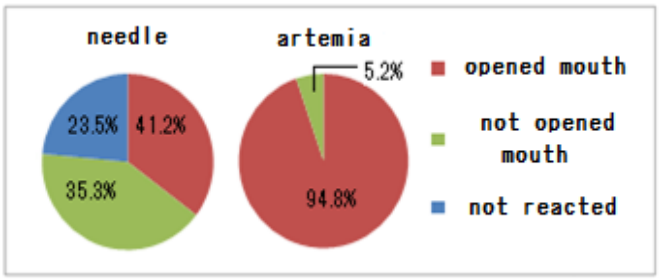

Figure 2. The response of polyps (proportions)

Figure 2 shows the responses of hungry (fasted for 10 days) and normally fed polyps. Approximately 38% of the normally fed polyps did not react, but almost all fasted polyps reacted. Furthermore, among those polyps that reacted, the fasted polyps opened their mouths more often than the normally fed polyps.

Figure 3. The reactions of hungry and fasted polyps (proportions)

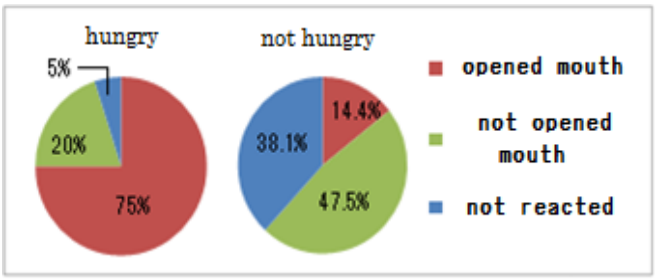

Figure 3 shows the response of the polyps to the glass beads.

In all, 80%of the polyps did not respond to the glass beads, and no polyps opened their mouths.

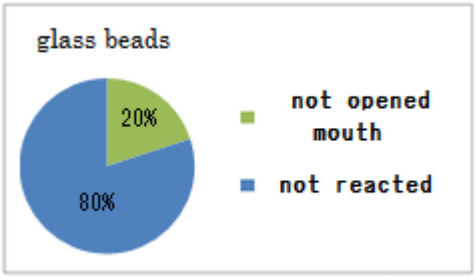

Figure 4. The responses of polyps to glass beads (proportions)

Figure 4 shows the responses of the polyps to the three solutions tested. Many polyps responded to peptone and to fermentum, whose principal ingredient is protein. However, no polyps responded to glucose, which is not a protein. In addition, as the concentration decreased, the number of polyps that responded to peptone decreased markedly, but the number of polyps that responded to fermentum decreased only very slightly.

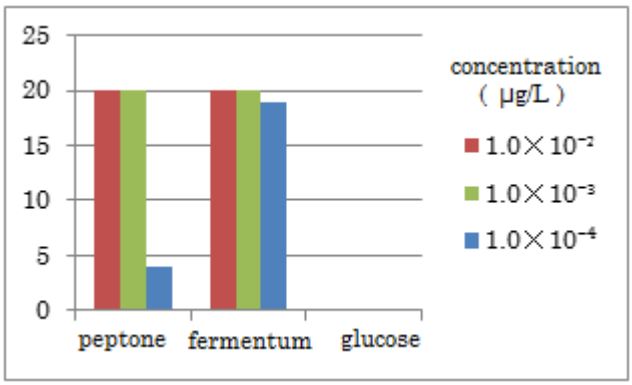

Figure 5. The reaction of polyps to each solutions

4. Discussion

All polyps reacted to Artemia. However, the reaction to the needle varied. Less than one-half of the polyps tested opened their mouths. This result does not indicate that the polyps clearly distinguished between food and non-food items. Figure 3 suggests that the fasted polyps responded more frequently to non-food items. Therefore, it is suggested that polyps can distinguish food from non-food items to a certain extent and that this ability is affected by the level of hunger.

In the first experiment, a needle was used because it is easy to touch the tentacles of the polyps with a needle. However, the impulse produced by exposure to the needle is stronger than that produced by exposure to Artemia. Therefore, it cannot be determined whether polyps distinguish food from non-food items physically or chemically. It is also possible to interpret "reacted but did not open mouth" as a defensive response. Figure 4 shows that the number of polyps that responded to the glass beads, which have a more Artemia-like impulse than the needle, was less than the number of polyps that responded to the needle. Figure 5 suggests that the polyps react to amino acid and protein in the absence of any physical impulse. Therefore, it appears that the polyps distinguish food from non-food items chemically rather than physically. This finding is consistent with Fulton's result that *Cordylophora* distinguishes food from non-food items based on chemical cues.

In addition, Figure 5 indicates that *Aurelia* polyps respond to certain amino acids because both solutions that produced responses contained components of membranes. Membranes contain proline. Therefore, these results suggest that *Aurelia* polyps react to proline.

In this experiment, the amino acid that is the trigger of the polyps' feeding behavior could not be identified. To identify this amino acid, it is necessary to expose the polyps to different amino acids to find the amino acid that triggers the polyps' feeding behavior.

5. Conclusions

This study shows that *Aurelia aurita* polyps distinguish food from non-food items based on chemical cues and that the key substance involved in this response is protein. A comparison of these results with the results of Fulton's experiment shows that *Aurelia aurita* and *Cordylophora* use different substances to identify food. To demonstrate this outcome, it is necessary to conduct experiments in the same way that Fulton conducted his experiments. It is also expected that information about the mechanism of the polyps' responses to food will contribute to research on the evolution of impulse receptors.

6. References

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Flower Fairy *E.coli*

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Abstract: Flower Fairies are imaginary creatures existing in fairy tales that can make various flowers bloom. In addition, such a concept, their magical power, would be useful in agriculture. In this study, we aimed to create a Flower Fairy using *E. coli*, a bacterium widely used in the field of biotechnology. To this end, we focused on the FLOWERING LOCUS T (FT) protein, a main factor in flower formation, and the R9 peptide, which mediates the intake proteins. We were able to produce the FT protein in *E. coli* and assess the function of the R9 peptide. As a result, we obtained an *E. coli* system to produce FT protein to encourage flower formation.

Keywords: Flower Fairy *E. coli*, FT protein, R9

1. Introduction

Flower Fairies are imaginary creatures existing in fairy tales that can make various flowers bloom, and such a concept would be useful for agriculture. In this study, we aimed to create a Flower Fairy using *E. coli*, a bacterium widely used in the field of biotechnology. Our goal was to generate a new *E. coli* as 'Flower Fairy *E. coli*' and induce flower formation by its application to leaves.

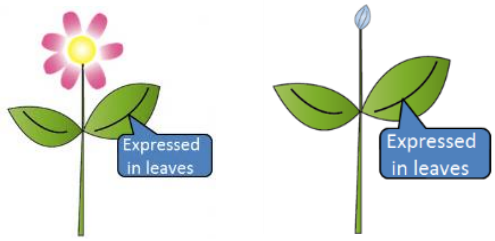

Figure.1 FT is produced in the leaves and is transported go to the stem apex to activate flower formation.

We first focused on the FLOWERLING LOCUS T (FT) protein from *Arabidopsis thaliana*. The FT protein is a type of plant hormone called Florigen. In plants, the FT protein is produced in the leaves and transported to the stem apex where is functions (Fig. 1) to up-regulate the genes involved in flower formation.

We constructed an FT-producing system by introducing the gene into *E. coli*.

However, as the FT protein cannot traverse the cell membrane, the nona-arginine peptide (R9 peptide) was utilized for plant cell uptake. The peptide is composed of nine arginines and has an overall positive charge in water.

Our system based the FT protein and R9 peptide has the potential to transform a fairy tale into reality.

2. Methods

2.1. Main experiment

(1) Polymerase Chain Reaction

PCR using ToYoBo KOD FX or ToYoBo KOD PLUS

Table1. For the use of KOD plus ver2

25 mM MgSO4	3 μL
2 mM dNTPs	5 μL
10x Buffer for KOD plus ver.2	5 μL
Template DNA (5 ng/μL)	5 μL
Forward primer (10 μM)	1.5 μL
Forward primer (10 μM)	1.5 μL
KOD plus ver.2	1 μL
MilliQ H_2O	28 μL
Total	50 μL

Table2. For the use of KOD FX

2 mM dNTPs	10 μL
2x Buffer for KOD FX	25 μL
Template DNA	5 μL
Forward primer (10 μM)	1.5 μL
Forward primer (10 μM)	1.5 μL

KOD FX	1 μL
MilliQ H_2O	6 μL
Total	50 μL

・Template DNA dilution. Transfer 1 μL of DNA at a concentration of 2-100 ng/μL to a clean tube and add 99 μL MilliQ H2O.

・Primer dilution. At a concentration of X μM, dilute the primer X times; transfer 1 μL to a clean tube and add 99 μL MilliQ H2O.

・Mix the following reagents:

・Incubated the mixture at 94℃for 2 mins.

・The PCR reaction: 25-40 cycles at 98 for 10 s, Tm-5 for 30 s, and 68℃for 1 min (1 min for 1 kb) (Tm is the temperature at which the primer will denature).

・Confirm amplification using agarose gel electrophoresis.

Colony PCR: Takara Ex Taq

・Mix the following reagents under sterile conditions:

Table3.

10x PCR buffer (TAKARA)	40 μL
2.5 mM dNTPs	8 μL
Primer 1 (10 pmol/μL)	8 μL
Primer 2 (10 pmol/μL)	8 μL
Ex Taq HS (TAKARA)	1.6 μL
MilliQ H_2O	334 μL (to a total of 400 μL)
Total	400 μL

・Dispense 25 μL into 15 tubes.

・Pick a single colony and transfer it to each tube.

・Suspend the colony.

・Incubate for 10 mins at 90℃

・PCR reaction: 35 cycles at 94℃for 30 s, 55 for 30 s, and 72 for 1 min.

・Hold at 72 for 4 min.

・Add 5 μL Loading Buffer to each tube.

・Confirm amplification using agarose gel electrophoresis.

・Negative Control: do not add a colony.

・Positive Control: add a colony that will yield a product using these primers.

(2) Preparation of competent cells

・Streak *E. coli* cells onto an LB plate; (BL21(DE3)LysS cells on LB plate+34 mg/ml chloramphenicol).

・Allow the cells to grow at 37 overnight.

・Place one colony in 10 mL LB medium (+antibiotic selection if necessary) and grow overnight at 37.

・Use 2 ml LB medium for an absorbance blank. Transfer 5 mL overnight DH5a culture into 500 mL LB medium in a 3 L flask.

・Grow the cells at 37 (250 rpm), until OD600= 0.4 (~2-3 hours).

・Transfer the culture to 2 centrifuge bottles (250 mL) and place on ice for 20 mins.

・Centrifuge the cells in a Sorval GSA rotor at 4 for 10 mins at 3,000 x g.

・Subsequent resuspensions may be performed in the same bottle. The cells must remain cold for the rest of the procedure. Transport the tubes on ice and resuspend the cells on ice in the cold room.

・Pour off the medium and resuspend the cells in 30 mL of cold 0.1 M $CaCl_2$. Transfer the suspended cells to 50 mL polypropylene falcon tubes and incubate on ice for 30 mins.

・Centrifuge the cells in a Sorval RT6000B rotor at 4 for 10 mins at 3,000 x g (2500 rpm)

・Pour off the supernatant and resuspend the cells (by pipetting) in 8 mL cold 0.1 M $CaCl_2$ containing 15% glycerol. Transfer 140 μL to (1.5 mL) Eppendorf tubes placed on ice.

・Freeze the cells in liquid nitrogen. Cells stored at -80 can be used for transformation for up to ~6 months.

(3) Miniprep

Use the QIAprep Spin Miniprep Kit Cat. No. 27104 from QIAGEN

・Pick a single colony from a freshly streaked selective plate and inoculate a culture of approximately 3 mL LB medium containing the appropriate selective antibiotic.

・Incubate at 170 rpm for 8 h at 37 with vigorous shaking.

・Transfer half of the culture to a tube.

・Harvest the bacterial cells by centrifugation at 14,000 x g for 1 min at 4℃ Remove the medium by pouring off.

・Transfer the other half of the culture to the same tube and harvest. Remove the medium by pipetting.

・Resuspend the pelleted bacterial cells in 250 μL Buffer P1 and mix thoroughly by pipetting.

・Add 250 μL Buffer P2 and mix thoroughly by inverting the tube gently 4-6 times.

・Add 350 μL Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times.

・Centrifuge for 10 min at 14,000 x g at 4.

・Apply the supernatants from step 10 to the QIAprep spin column by pipetting.

・Centrifuge for 10 s in a table-top microcentrifuge. Discard the flow-through fraction.

・Wash the QIAprep spin column by adding 0.5 mL Buffer PB and centrifuging for 10 s in a table-top microcentrifuge. Discard the flow-through fraction.

・Wash QIAprep spin column by adding 0.65 mL Buffer PeE and centrifuging for 10 s in a table-top microcentrifuge.

・Discard the flow-through fraction and centrifuge for an additional 1 min to remove the residual wash buffer.

・Place the QIAprep column in a clean tube. To elute DNA, add 50 μL water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

・Discard the QIAprep spin column.

・Measure the concentration of DNA by using Eppendorf BioPhotometer plus.

・Restriction Digestion.

・Confirm digestion using agarose gel electrophoresis.

・Use the Wizard Plus SV Minipreps DNA Purification System from Promega

・Harvest 1–5 ml (high copy-number plasmid) or 10 ml (low copy-number plasmid) of bacterial culture by centrifugation for 5 minutes at 10,000 x g in a tabletop centrifuge. Pour off the supernatant and blot the inverted tube on a paper towel to remove the excess medium.

・Add 250 μl of Cell Resuspension Solution and completely resuspend the cell pellet by vortexing or pipetting. It is essential to thoroughly resuspend the cells. If not already using a microcentrifuge tube, transfer the resuspended cells to a sterile 1.5 ml microcentrifuge tube(s).

・Add 250 μl of Cell Lysis Solution and mix by inverting the tube 4 times (do not vortex). Incubate until the cell suspension clears (approximately 1–5 minutes).

・Add 10 μl of Alkaline Protease Solution and mix by inverting the tube 4 times. Incubate for 5 minutes at room temperature.

・Add 350 μl of Neutralization Solution and immediately mix by inverting the tube 4 times (do not vortex).

・Centrifuge the bacterial lysate at maximum speed (approximately 14,000 x g) in a microcentrifuge for 10 mins at room temperature.

・Transfer the cleared lysate (approximately 850 μl, Section 3.B, Step 6) to the prepared Spin Column by decanting. Avoid disturbing or transferring any of the white precipitate with the supernatant.

・Centrifuge the supernatant at maximum speed in a microcentrifuge for 1 minute at room temperature. Remove the Spin Column from the tube and discard the flow-through fraction from the Collection Tube. Reinsert the Spin Column into the Collection Tube.

・Add 750 μl of Column Wash Solution, previously diluted with 95% ethanol, to the Spin Column.

・Centrifuge at maximum speed in a microcentrifuge for 1 minute at room temperature. Remove the Spin Column from the tube and discard the flow-through fraction. Reinsert the Spin Column into the Collection Tube.

・Repeat the wash procedure using 250 μl of Column Wash Solution.

・Centrifuge at maximum speed in a microcentrifuge for 2 minutes at room temperature.

・Transfer the Spin Column to a new, sterile 1.5 ml microcentrifuge tube, being careful not to transfer any of the Column Wash Solution with the Spin Column. If the Spin Column has Column Wash Solution associated with it, centrifuge again for 1 minute at maximum speed.

・Transfer the Spin Column to a new, sterile 1.5 ml microcentrifuge tube.

・Elute the plasmid DNA by adding 100 μl of Nuclease-Free Water to the Spin Column. Centrifuge at maximum speed for 1 minute at room temperature in microcentrifuge.

・After eluting the DNA, remove the assembly from the 1.5 ml microcentrifuge tube and discard the Spin Column.

・The DNA is stable in water without the addition of a buffer if stored at -20°C or below; the DNA is also stable at 4°C in TE buffer. To store the DNA in TE buffer, add 11 μl of 10X TE buffer to 100 μl of the eluted DNA. Do not add TE buffer if the DNA is to be used for automated fluorescent sequencing.

・Cap the microcentrifuge tube and store the purified plasmid DNA at -20°C or below.

3. Results

3.1. Synthesis of the FT protein in *E. coli*

We utilized *E. coli* to produce the FT protein by transforming an expression plasmid.

Because the FT sequence contains two restriction enzyme sites of enzymes used for cloning, we eliminated the cleavage sites by performing inverse PCR of the plasmids. We used two types of primers that contained one base mismatch between the primer and cDNA to mutate the FT protein. If plasmid had injure by some cause, plasmid would be always recovered the sequence by a polymerase's direction going only one direction, but using these type, the polymerase's tensile direction became reverse. We obtained a mutated FT sequence that cannot be cleaved by EcoR1 or Pst1 thus facilitating cloning through the use of these enzymes.

We added a T7 promoter regulated by IPTG to drive the expression of the FT gene.

To confirm the specific expression of the FT protein, we performed western blotting using goat anti-FT protein antibody.

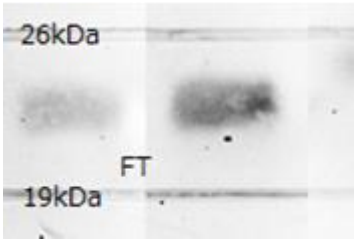

Figure 2. Verification of expression of FT protein in *E. coli* by western blotting.

The cells were precultured overnight and diluted into fresh SOC medium. IPTG was added when the OD600 was approximately 0.5, and the cells were incubated for 4 h at 20°C; 100 μL of the culture was used for SDS-PAGE.

Lane 1: FT cell lysate, not induced

Lane 2: FT cell lysate, IPTG induced

3.2. R9 peptide were able to let cells intake proteins

The addition of the R9 peptide enabled the cells to take up the FT protein, the function of which is to upregulate the transcription of proteins leading to flower formation. Therefore, the FT protein expressed in *E. coli* must be able to enter plant cells to induce flower formation, which can be achieved using nona-arginine peptide (R9 peptide), a type of cell-penetrating peptide (CPP).

The following is the mechanism of the R9 peptide-FT protein chimera (Fig)

1. The R9 peptide adheres to the cell membrane because of the hydrophobic character.
2. The cell responds to the stimulus, causing macropinocytosis, a specific form of endocytosis.
3. The protein in the invaginating region of the cell is then taken into the cell.

In this study, we performed an experiment to confirm this function of R9 using *Arabidopsis thaliana* leaves. We scratched the cuticle of the Arabidopsis leaves and soaked them into a solution of GFP and R9 or only GFP.

After 5 minutes, we washed cells with PBS to remove GFP and the R9 peptides from the sample surface. Using Hoechst stain, we observed the samples using confocal microscopy.

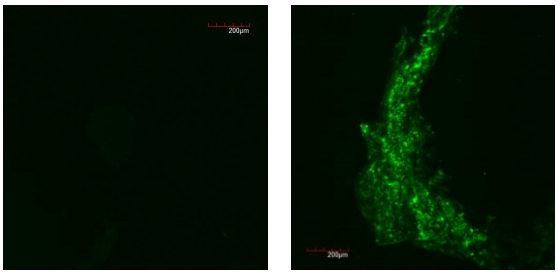

Figure 3. Verification of R9 function using GFP.
Left: Inside cell without R9
Right: Inside cell with R9+GFP

Figure 3. shows the cells of *Arabidopsis thaliana* leaves soaked in GFP solution for five minutes, followed by Hoechst staining. The samples in the left panel were soaked in only GFP, whereas the samples in the right panel were soaked in GFP and the R9 peptide. GFP is clearly inside the cells when the R9 peptide is included.

4. Discussion

In this report, we showed that *E. coli* can express the FT protein by western blotting and that the R9 peptide can mediate plant cell absorption of the protein through the visualization of GFP. Previous studies indicate that smaller target proteins are more readily transported by the R9 peptide. FT is a smaller protein then GFP, thus it seems likely that the R9 peptide has the ability of delivering the FT protein.

5. Conclusions

Based on our results, our Flower Fairy *E. coli* should be able to promote flowering. Although additional experiments are required, for example, confirmation of the activity of the FT protein in plant cells, the result of previous studies strongly suggests that Flower Fairy *E. coli* induce flowering.

The FT protein is a eukaryotic protein and may require post-translational modifications, which are important for function in eukaryotic cells. However, the mechanism of transcriptional regulation by FT has been elucidated, and it appears that post-translational modifications are irrelevant.

In particular, Washida indicates that the FT-like protein of rice produced in *E. coli* was able to function in plant cells after introduction using the R9 peptide.

Although further study is required, previous research indicates that the function of the partner protein is not affected by the presence of the R9 peptide. Therefore, we suggest that our Flower Fairy *E. coli* has the ability to induce blooming.

Our system based the FT protein and R9 peptide has the potential to translate a fairy tale into reality.

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How “romantic love” has affected the modern family in Japan

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Abstract: It might be said that the modern family is a result of an emphasis on the value of “romantic love”. Presumably, there is a close connection between “romantic love” and the modern family. This study investigated how the change in the value of love has affected the system of the modern family. To address this issue, the novels of Natume Souseki and Murakami Haruki were analyzed. In Souseki’s works, in loving a person, the features of self-consciousness, self-reflection, responsibility and a lack of sexual desire are the highest priorities. However, in Murakami Haruki’s works, these values have been lost, and loving the entire person is the goal, as a substitute no longer exists. From a sociological perspective, the diversity of self-consciousness can be thought of as a weakness of self-consciousness. Although the systematic form of the modern family may no longer exist, the “self” and the new values that have emerged might be more flexible.

Key Words: modern family, romantic love, self-consciousness(“self”), self-reflection, responsibility, sexual desire, the dualism, the fragment of self-consciousness, the diversity of self-consciousness, human relationships

1. Introduction

Recently, it has been noted that problems related to family affairs have become more controversial. Although it is difficult to say what situations might be called dysfunctional, it is clear that the Japanese family is different from what it used to be. The stereotype of the Japanese family is the Sazae-san family, which is the subject of one of the most popular Japanese comics, created by Hasegawa Matiko. The Sazae-san family works functionally. For example, all the members eat meals sitting around the round table, and grandpa, Isono Namihei, represents a role model for the children and he helps his daughter Isono Sazae and her husband, Isono Masuo. The members of this family connect harmoniously with each other.

However, it seems that this model of the family has changed. Mita Munesuke, a sociologist and a professor at Tokyo University, states that the turning point was the period of the 1970s-1980s (Mita,2006,[1]). A clear change can be seen in the presentation of the family in many movies and dramas. For example, in the movie “Kazoku Game” (Family Game) by Morita Yoshimitu, the members of the family no longer sit like the Sazae-san family. Instead, they sit in a line, and there is no lively conversation.(Mita,2006,[1]) In the work of Yamazaki Tetu, family conversations are described as meaningless(Mita,2006,[1]). According to these examples, it is clear that the modern family has changed.

One survey showed that increasing numbers of Japanese people are choosing not to get married(Ito,1996,[2]). This may indicate that more people now think of creating a family as a meaningless enterprise. This phenomenon may also indicate that the value of love has changed; the original concept of the modern family was closely associated with the value of love, especially in Japan.(Mita,2006,[1])

However, few studies on the relationship between love and the modern family have been conducted. In this study, I investigated whether romantic love was closely related to the modern family; indeed, “romantic love” is described as a driving force for the creation of the modern family(Kawai,2011,[3]).

Here, I mainly attempted to identify the transition in the value of love in Japan from modern times to post-modern times. For this purpose, I attempted to interpret the work of two Japanese novelists, Natume Souseki and Murakami Haruki. These authors were selected because they differ in how their novels describe romantic love. Furthermore, Natume Souseki represents the modern period, and Murakami Haruki represents the post-modern period.

2. Overview

Three steps were employed in the current investigation.

First, according to the concept of the modern fami-

ly proposed by Mita Munesuuke and the concept of "romantic love" proposed by Kawai Toshio, I attempted to analyze the modern family and the value of love.

Second, I attempted to interpret several of the novels written by Natsume Soseki (1867-1917) and Murakami Haruki (1949-). The novels by these authors provide ideal case studies for understanding the value of love. In some cases, these novels provide contrasting versions of the value of love. Thus, these novels might provide an understanding of how the value of love has changed. This is the main question that this study sought to address.

Third, I attempted to analyze how the change in the role of romantic love has influenced the modern family in Japan.

Finally, I attempted to demonstrate that the concepts of self-consciousness and sexual desire are at the core of the change in the value of love. In negative points, it is workless of modern family. However, in positive points, it can be thought of the diversity of self-consciousness.

3. Result and Discussion

3.1. Modern Family

Here, I provide a description of the modern family. The modern family is a system that maintains the "self", or self-consciousness. The concept of the modern family emerged after the old-fashioned construction of the "extended family" deteriorated and the family was reconceptualized with its re-united.(Mita,2006,[1]) There are two explanations for the creation of the modern family.

First, Kawai Toshio says that, with respect to Western history and culture, individuals must unite with partners to become couples as a result of individualism, which is born out of separation from God and traditional communities. After the "self" has emerged, individuality becomes more complicated. That is to say, individuality is defined through relationships (Asami,2001,[4]). However, the connection with God or an extended community has disappeared, which had defined each persons from above. This is the definition of the "persona". As a result, persons are forced to create new relationships by themselves to protect the "self"; this separation causes people to feel lonely (Kawai,2011,[3]). Loneliness disrupts the "self". Thus, people will create new relationships with others to maintain the "self" and relieve the feeling of loneliness. Therefore, modern family can be observed as a system to establish the "self".

Second, the way in which the modern family has been established is deeply connected to the development of capitalism. The traditional family was not acceptable to capitalism. Thus, the traditional family unit known as the "extended family" was dissolved to create a consumer-unit (Mita,2006,[1]).

As a result, under the new values, the members of a family are re-united to develop into a modern family.

3.2. New values: "Romantic Love"

A new value known as "romantic love" was introduced as a driving force for re-union. Here, I describe the concept of "romantic love". This concept was proposed by Kawai Toshio. According to the concept of "romantic-love", people are expected to eagerly seek a romantic partner to relieve the feeling of loneliness. It is not always necessary for two people's love to become real, and sexual desire is not an essential component of romantic love. The necessity of self-consciousness brings about a strong sense of self-control or responsibility. (Kawai,2011,[3]). This is an especially interesting point; it is difficult, and perhaps impossible, for us to separate sexual desire from love. This is because there is a strong correlation between love and sexual desire. (Asami,2001,[4]) In my investigation of the literary accounts of love, I found that love was often associated with sexual desire. For example, consider *The tale of Genji*. In terms of "romantic love", *The Tale of Genji* is not considered a "romantic love" story. Asami Katsuhiko states that because of sexual desire, romantic love is the most threatening to the "self". Thus, sexual desire was excluded from love by introducing the concept of dualism. (Asami,2001,[4])

Thus, "romantic love" can be seen as a type of ideology that has emerged for two reasons.

First, loving someone can relieve the feeling of loneliness. (Kawai,2011,[3]) In *Kokoro* by Natume Souseki, Sensei suffered from loneliness and did not trust anybody before he meet his wife (Natume,1908,[5]) In *Kafka on the Shore* by Murakami Haruki, Kafka, a fifteen-year-old boy, feels lonely and seems to try to find his mother or his sister during his journey. (Murakami translated by Phillip,2002,[6])

These works depict persons who are alone and in need of connection with others, which means that they need to find love. It is important to reiterate that the "self" depends on the presence of other people.(Asami,2001,[4]) Thus, the "self" is likely to be upset and symbolizes a weak constitution so that sexual desire is excluded from love.

Second, "romantic love" requires that people should be responsible for loving others. During the course of the industrial revolution, people became separated from larger communities, such as farming villages. A person no longer felt connected to a large community (Kawai,2011,[3]). Prior to the industrial revolution, people lost their ties with God due to the Reforma-

tion. That is to say, there was no actual feeling of connection between people and their God or community. As an historical observation, it seems that the concept of "romantic love" was created hastily. Therefore, the "self" is required to ensure its existence. This attitude has brought about the notions of self-reflection and responsibility.

These separations have forced people to solidify the concept of the "self". As a result, people have come to observe themselves, leading to self-reflection. In addition, the sense of responsibility has been strengthened through this process, as the division between "romantic-love" and sexual desire became more pronounced.

Therefore, according to the concept of romantic love, people are expected to become entirely involved with those that they love at the expense of their own identities, without sexual desire, as required by the concept of responsibility. (Kawai,2011,[3]).

A typical example of this is William Shakespeare's *Romeo and Juliet,* written in the seventeenth century. In this story, the love between Romeo and Juliet did not became real until their deaths. In *Norwegian Wood*, written by Haruki Murakami, Watanabe (one of the main characters) is eager to seek Naoko, who is his lover. Her death causes his consciousness to drift. (Murakami,2000,[7]), and the fact that his love is not reciprocated is emotionally painful for him. (Jay,2000,[8]). In this case, the relationship between Naoko and Watanabe is filled with sexual interaction. However, the way that Watanabe interacts with Naoko also demonstrates responsibility and self-reflection.

Therefore, romantic love presumably has encouraged lonely persons to create a solid system known as the "modern family" to solidify the "self". As a result, love is divided between sexual desire and "romantic love". (Asami,2001,[4]), and "romantic love" is considered a preferable pursuit.

3.3. The change in "romantic love": Interpreting Natume Souseki and Murakami Haruki – Understanding love in modern times

The works of Natume Souseki were employed to understand love in modern times. Souseki is one of the most famous novelists who represent modern Japan. His stories describe the values, things and people in the Meiji and Taishou eras.

The Meiji era corresponds to the birth of modern Japan. In 1868, the Meiji restoration occurred to end the Tokugawa shougunate. Under the Emperor Meiji, the new government modernized Japan by doing away with the feudal system (Bakuhan Taisei), organizing the Imperial Army and Navy and boosting industry in Japan's. These reformations were undertaken very quickly. Thus, his novels clearly express the thoughts of modern times. An account of modern love is given in Sanshiro.

Sanshiro encountered a maid in an environment that reminded him of his homeland. In Nagoya, he happened to stay in the same room of the same hotel with this woman. She tempted him sexually; for example, she tried to bathe with Sanshiro when he was taking a bath. When Sanshiro went to bed, the woman slept with him in the same *futon*. Sanshiro was emotionally affected by her, but he managed to avoid having an intimate relationship with her. (Natume,1907,[9])

He should never fallen apart like that. His education counted nothing here .It was all a matter of character. He should have done better. But if women were always going to behave that way, then he as a educated man would have no other way to react-which meant that he would have to steer clear of them. It was a gutless way to live and much to constraining as though he had been born some kind of cripple. And yet …..(Jay, 2009,[10])

This is a typical example of "romantic love". In the end there is no sexual relationship between Sanshiro and the maid. However, his mind reveals this conflict, and he his uneasy. This situation is caused by his self-consciousness and his sense of responsibility. (Kawai,2011,[3]) The temptation that she presents perturbs his self-consciousness. Therefore, he is troubled by identifying himself comprehensively. This conflict symbolizes the "self" as inflexible and a source of weakness. We must identify ourselves in relationships, which are diverse and variable. However, the "self" is less flexible than we expect. Thus, we sometimes try to create a stable and fixed situation by possessing the lover. With regard to the process of possession of the lover, love can be observed as an attempt to possess the lover's consciousness. On the other hand, sexual desire refers to an attempt to possess both the lover's consciousness and the lover's body. Through these acts of possession, the "self" is supposed to become a solid constitution. However, sexual desire is sometimes dangerous to the "self" so that separation is needed to protect the "self". Thus, love can be thought as a defense mechanism. (Asami,2001,[4]) In Sanshiro's case, his strong sense of responsibility and his shallow relationship with the maid prevented him from connecting with her. Therefore, he protected his "self" by remaining alone.

In romantic love, it is important to associate the person you love with your self-conscious and your sense of responsibility by avoiding sexual desire.

Another important aspect of loving someone is to love the entirety of the person or to be completely satisfied by. In *Kokoro,* Sensai says

Sensei once confined to me, "I have only ever

known one woman in my life. No one besides my wife has really ever appealed to me as a woman. And likewise for her, I am the only man. Given this, we are the happiest couples." (Meredith,2010,[11])

Therefore, in "romantic love", love requires self-consciousness and responsibility instead of sexual desire because it is important to love one's lover and need no substitute.

3.4. Love in post-modern times.

I also considered the novels of Murakami Haruki's to investigate how romantic love has changed in post-modern times. Murakami Haruki represents post-modern literature. Here I used the word "post modern" in reference to the time in Japan after the modern time. That is to say, I am referring to the period from after the 1970s to the present. Changes in literature and sociology are clear evident during this time (Mita,2006,[1]). Actually, the word "post modern" is highly controversial. Scholars have offered definitions of this term in variety of fields. However, the definition of the word "post modern" is not a main purpose here. Therefore, I have not tried to define the term post modern, and I do not use the word as a technical term. In his novels, it is presumed that self-consciousness and responsibility no longer exist. Compared with modern literature in Japan, sexual relationships are depicted vividly.(Kawai,2006,[3])

In *Norwegian Wood*, Watanabe has sex with women, but he does not feel guilty, and he experiences no conflict similar to that of Sanshiro.(Kawai,2011,[3])) In *Sputnik Sweetheart* by Murakami Haruki, the narrator does not hesitate to have sex with a woman he encounters during his trip. After they part, no connection remains between them. (Murakami,1999,[12]) Therefore, the concept of responsibility, which was critical in "romantic love", has been lost.

Self-consciousness is also lost. *Sputnik Sweetheart* provides a typical example Miu saw herself having sex with a man from a Ferris wheel. (Murakami,1999,[12]) This phenomenon is depicted as a fragment of self-consciousness. (Kawai,2011,[3]) In *Norwegian Wood*, Naoko says, "If I relaxed for a second, I would fall apart."(Jay,2003,[13]) The fragmentation of the self-conscious is often noted in discussions of post modern concepts.(Kawai,2011,[3])

Because of this fragmentation, it is impossible to desire the entirety of the person that one loves. Thus, in Murakami Haruki's works, some characters desire parts of another person, which results in an increasing emphasis on the physical aspects of a person.

In *Norwegian Wood*, Naoko seeks Watanabe's arms, not Watanabe himself.(Jay,1997,[14]) In *The Wind-Up Bird Chronicle,* Kasahara Mai seeks men's naked heads.(Rubin,1999,[15])Naoko and Kasahara Mai never love the entirety of one specific man.

Considering that these phenomena emphasize sexual relationships and the more physical aspects of love, I argue that the values of modern times no longer work. These changes in novels reveal that sexual desire has been connected with love again. In dualism, the mind is supposed to surpass body. However, this is evidently impossible, as "romantic love" cannot surpass sexual desire. Thus, dualism is worthless, and people cannot suppress their sexual desires for those that they love. Several data support this argument. According to surveys conducted by the sociologist Yajima Masami's, 68.3% of junior high school students and high school students believe that they are free to have sex with anybody under any conditions. Furthermore, almost 50% of junior high school students and high school students value appearance more than intelligence or kindness. Masami also states that sexual relationships are more common before going out.(Aditor Chuo University,2006,[16]) These findings provide evidence that physical aspects are more important than dating. Usami Takeshi indicates that the recent trend of love in Japan, which is called the "jyunai boom" (the pursuit of pure love), represents a counter-force against changes the modern idea of love.(Aditor Chuo University,2006,[16])

From another perspective, according to a survey on mental diseases in Japan because the 1990s, the prevalence of developmental disorders has increased. (Kawai,2011,[3])

Therefore, the concept of love has changed. It seems that the values of love have returned to the values of love in pre-modern time. This change has involved the loss of self-consciousness and responsibility. Kawai Toshio says these patients feel less guilty and experience less mental conflict, which is represented in Murakami Haruki's novels. (Kawai,2011,[3]) Therefore, it might be the case that the concept of "self", or self-consciousness, is also worthless. This fragmented self-consciousness might be a modern reality. It might result in the worthlessness of the modern family. However, from a sociological perspective, there is some notion of the future of family and self-consciousness.

3.5. Self-consciousness and the reality of the family from a sociological perspective

I have argued that the modern family has become dysfunctional. Some sociologists have investigated the reality of the modern family, arguing that the modern family is workless. However, others have suggested that the problem of the modern family is not as serious. After all, the modern family is only a system. Looking back on human history, many systems have emerged and then collapsed. What is more important than the system of the modern family is

nature of human relationships. In terms of human relationships, the workless of modern family could be considered as positive, and some sociologists have argued this point. One of them is Matsuda Misa, who is a professor of sociological intelligence at Chuo University. She has investigated communication between family members through the use of mobile phones and notes that the method of communication has changed. She says that the quality of "talkativeness" and "the diversity of self-consciousness" are is characteristics of family today (Aditer, Chuo University,2006,[16]) Generally speaking, "talkativeness" does not indicate a dysfunctional modern family. However, it is actually a factor of the family problem. This is because the members choose the person with whom they will talk, which means that the conversations among all family members are not always lively and active. Thus, it is thought that individual close relationships are the highest priority in the field of family sociology. This hypothesis relates to "the diversity of self-consciousness" because we must change our character according to the person with whom we are speaking. Furthermore, Mita Munesuke states that the center of the modern family has been lost, resulting in "the separation of love". (Mita,2006,[1]) Therefore, the modern family is certainly workless as whole because some members are excluded from intimate relationships. In terms of modern concepts or modern values, it is a bad thing because the system known as the modern family no longer works and the diversity of self-consciousness could be thought of as the fragmentation of self-consciousness.

However, human relationships are variable and flexible, and modern concepts are not realistic. Regarding the situation among the values of love, the lack of a center of self-consciousness and modern family is more natural. In my opinion, the core of these problems is that people worry about their involvement with other people. This anxiety has been a main theme of human philosophy, among other disciplines. I need more time to consider these problems.

4. Conclusions

Based on the interpretation of the works of Murakami Haruki and Natume Souseki, it is clear that the modern family has changed.

In *Kokoro,* although the "I" in the story feels out of place in his family, there is a sense of connection with his family members.(Natume,1914,[33]) This is because there is some detailed description of his family provided in the story.

However, in Murakami Haruki's novels, there is no longer any connection to family. For example, *in Kafka on the Shore*, the main character has few memories of his mother or his daughter. Furthermore, there are no descriptions of his family. During his journey, he meets several women and assumes that they are his daughter or his mother. He feels no emotional connections, such as love, anger, or hate and so on. Kawai Toshio states that the notion of the family has become a complete fiction. (Kawai,2011,[3]) From a literary perspective, it is clear that the values of love have changed and that the modern family has changed. The core of these changes might be changes related to the "self". How we understand and evaluate these changes is important because we cannot stop these changes. We must reconsider how we relate to others. From a sociological perspective, these changes are not necessarily negative, although Kawai Toshio and some other scholars appear to consider them as negative. In this paper, I posited that the "self" is a weak institution. However, due to the development of new communication tools or new values, we are able to communicate with others more freely and more flexibly. Thus, it might be possible to solve the problem of the "self". That sexual relationships are increasingly common might be a sign of these changes. It is likely that the "self" does not need to be limited by systems such as the modern family. Further studies are needed to determine what role the "self" may play in modern family and love.

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Prospect of Cancer Stem Cell-targeting Therapy

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Abstract: The Cancer Stem Cell model, which was proposed in the 1970s because of the wide heterogeneity in malignant tumors, has been validated for some cancers, including breast cancer and acute myeloid leukemia since the late 1990s. Cancer stem cells (CSCs) have both self-renewal activity and the ability to produce various differentiated cancer cells. They are resistant to chemo- and radiotherapy and have the capacity for invasion and metastasis, which can lead to recurrence. Therefore, a considerable number of researchers have been seeking novel treatments that target CSCs. In this review, I explain two existing CSC-targeting therapies – differentiation therapy and CSC niche-targeting therapy – and an emerging therapy by direct reprogramming, finally referring to the prospect of CSC-targeting therapy in the future.

Key Words: cancer stem cell, CSC-targeting therapy, direct reprogramming

1. Introduction

Previously, it was thought that all cancer cells were similar, but in recent years, they have been found to have phenotypic and functional heterogeneity. These heterogeneity most likely results from three factors: genetic mutations, epigenetic changes, and interactions with the microenvironment (Lindeman and Visvader, 2012). However, cancer cells have such wide heterogeneity that the reasons mentioned above cannot fully explain it. Therefore, it has been assumed that there is a hierarchical organization of cells that results in the phenotypic and functional heterogeneity. This assumption led to the cancer stem cell model. Recently, stem cell-like cells, which have the ability to proliferate and differentiate into various types of cancer cells, have been found in many cancers, including breast cancer (Al-Hajj et al., 2003), acute myeloid leukemia (AML) (Bonnet and Dick, 1997; Lapidot et al., 1994) and glioblastoma (Bao et al., 2006). Therefore, it can be said that the CSC model has been validated using various types of cancers. According to the definition of the American Association for Cancer Research, CSCs exist within tumors and have both self-renewal activity and the ability to produce various origin-and-type-specific cancer cells that construct the tumor. They are resistant to chemo- and radiotherapy, which greatly contributes to cancer recurrence. Like normal stem cells, CSCs show a high degree of therapeutic resistance; for example, CSCs express various ABC transporter pumps that export drugs out of the cell, and they also have a very efficient DNA repair system. Finally, they rarely divide and are in a state of long-term quiescence in their niche, called dormancy. If only non-cancer stem cell (nCSC)-targeting drugs, which have been primarily used in the clinic, are administered, CSCs will circumvent them, leading to recurrence within years or decades. Therefore, a considerable number of scientists have been seeking a novel treatment to target CSCs. In this review, I first discuss existing therapies targeting CSCs, such as differentiation therapy and CSC niche-targeting therapy. I will then introduce a novel emerging therapy in which CSCs are directly reprogrammed to normal cells by the introduction of a certain type of transcription factor.

2. Existing CSC-targeting therapy

2.1. Differentiation therapy

Considering the notorious hallmarks of CSCs that are caused by their undifferentiated state, it is easily expected that CSCs will become less malignant if they are forced to differentiate into mature cells. Some researchers have been seeking a novel treatment to induce CSC differentiation. Human myeloid leukemia cells (HL60) are in an undifferentiated state without differentiating into mature blood cells. Some studies have shown that all-trans-retinoic acid (RA) induces the differentiation of HL60 cells both in vitro and in vivo, which makes a significant contribution to the improvement of the mortality rate caused by the disease. In addition, bone morphogenic proteins (BMPs) play a key role in inducing glial differentiation in glioblastomas and inhibiting tumor growth (Piccirillo et al., 2006).

2.2. CSC niche-targeting therapy

As mentioned, CSCs are resistant to chemo- and radiotherapy. CSC niches can lead to recurrence. The niches contain specific mesenchymal, vascular, immune, inflammatory and stromal support cells (Wag-

ers, 2012). They also protect CSCs from chemo- and radiotherapy and help CSCs maintain an undifferentiated state. Within a niche, CSCs rarely divide and are in a state of long-term quiescence called dormancy. The niche serves as a "shield" for CSCs. Therefore, some scientists have hypothesized that CSC activation by niche destruction may cause them to become less resistant to chemo- or radiotherapy. In the research of some hematological malignancies, such as myeloid leukemia (AML) and chronic myeloid leukemia (CML), several studies have revealed agents that can activate dormant/quiescent hematopoietic stem cells (HSCs) (Trumpp et al., 2010), including some cytokines such as granulocyte colony-stimulating factor (G-CSF) and (IFNα). If HSCs are activated, it is likely that they will respond well to chemo- or radiotherapy. As is widely known, IFNα plays a pivotal role in the regulation of resistance to viral infections. Moreover, it has been shown to induce activation of dormant HSCs (dHSCs). As soon as it is administered, IFNα binds to its receptor on HSCs, resulting in rapid STAT1 phosphorylation and the transcription of a set of IFNα target genes (Figure 1). In addition, IFNα induces the over-expression of stem cell marker Sca-1 on the surface of activated HSCs, which may enable stem cell-targeting drugs to detect HSCs. This suggests that IFNα can be used for CSC-targeting therapy; for example, to activate quiescent CSCs in a niche, IFNα is administered, followed by the administration of a certain CSC-targeting agent. This two-step administration may eradicate CSCs, which make a considerable contribution to recurrence.

3. Direct reprogramming

In 2006, Yamanaka and his colleagues succeeded in inducing pluripotent stem cells by introducing four factors (Sox2, Oct3/4, Klf4 and c-Myc) into mouse fibroblasts (Yamanaka et al., 2006). This discovery spawned three novel research fields (Figure 2), including direct reprogramming, in which a certain cell is directly reprogrammed to another type of cell by introducing certain factors into the cell. In this manner, functional platelets were induced from mouse and human fibroblasts by p45NF-E2/Maf (Ono et al., 2012). Using this analogy, I hypothesize that CSCs can be directly reprogrammed to normal cells by introducing certain factors that are expressed in normal cells. If this approach can be applied to CSC-targeting drugs, then it would become a promising cancer treatment method. To test the validity of my hypothesis, I performed a literature review and found an article featuring the novel therapy of direct reprogramming. Mori and his colleagues introduced Yamanaka factors into cancer cells and generated induced pluripotent cancer cells, which assumed much less malignant features (Mori M et al., 2012). Because this method has the possibility in induce tumor development, they made another attempt to introduce the defined factors using microRNA, which is a very safe and efficient way of reprogramming a cell. Based on these studies, I hypothesize that this technique can be applied to CSCs. The authors did not attempt to reprogram CSCs, only cancer cells. I expect that there is a more efficient method by which CSCs can be reprogrammed with factors that induce transformation into normal cells. It is true that this method also carries the possibility of developing a tumor because cancer cells have genome instability. However, some studies of cancer cell reprogramming have reported that the cells do show less-malignant features (Matushansky et al., 2012). Therefore, it is possible that CSCs may be reprogrammed to less-malignant cells, which may enable us to devise a novel cancer therapy.

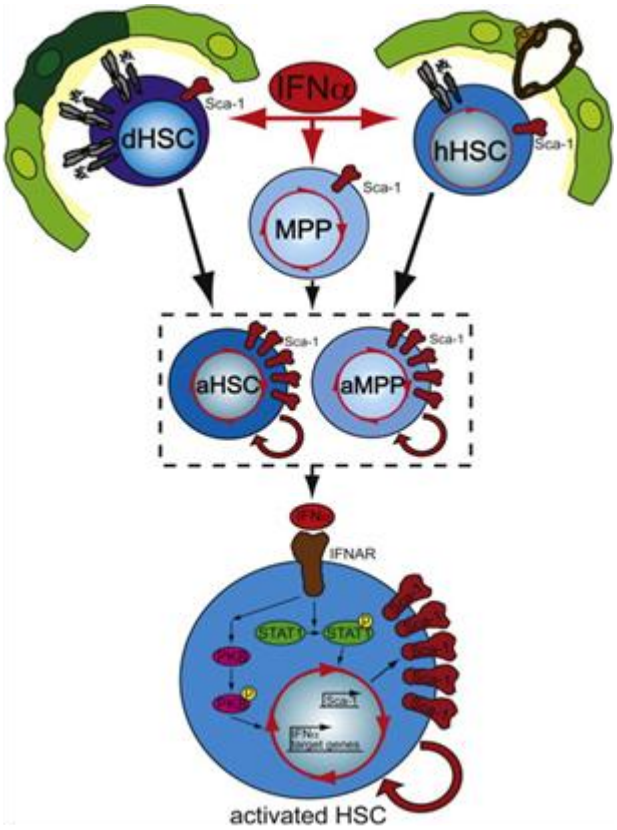

Figure 1. IFNα induces HSC activation in vivo
This figure is cited from Andreas Trumpp et al. (2010)

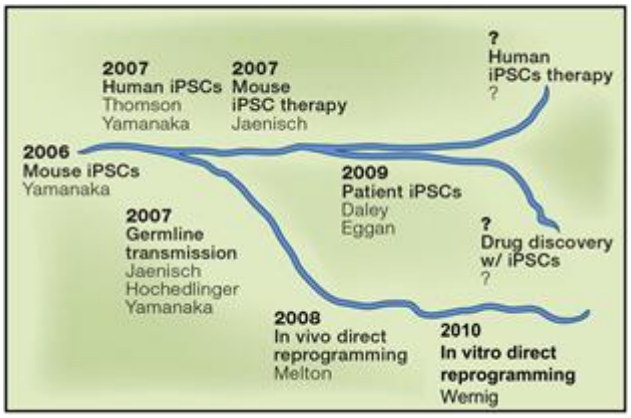

Figure 2. New Scientific Streams that Emerged from the Development of iPS cells
This figure is cited from Yamanaka S. (2012)

4. Conclusions

In conclusion, CSC-targeting therapies hold great potential for eliminating all cancer cells because CSCs play an essential role in invasion, metastasis and recurrence. The treatments presented in this review, particularly direct reprogramming, may provide novel CSC-targeting therapies. However, in some cancers, the CSC markers that distinguish CSCs from nCSCs are unknown. In fact, in other cancers, no hierarchical epigenetic difference has been observed, which implies that they do not follow the CSC model. The clear difference between CSCs and nCSCs remains to be elucidated. Therefore, to firmly create a novel protocol targeting CSCs, further research on the characteristic markers of CSCs that discriminate CSCs from nCSCs will inevitably be needed. Thereafter, a precise drug delivery system in which the drugs mentioned above can be correctly delivered to the target cells will need to be introduced. Thus, eradicating cancer will become a possibility.

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The Role of Oxygen in Bincho Charcoal Battery Cells

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Abstract: Because global warming has recently become an international concern, we need to identify new, clean energy sources. One of these clean energy sources is the bincho charcoal battery cell. The bincho charcoal battery cell is a new type of battery that generates electrical energy with bincho charcoal. Although we have determined that the battery can generate energy without emitting carbon dioxide, it is not yet ready for practical use. To improve the battery performance, it is necessary to clarify how it works. Previous studies have given the following formula:

cathode (O_2) $3O_2 + 6H_2O + 12e^- \rightarrow 12OH^-$

Anode (Al) $4Al \rightarrow 4Al^{3+} + 12e^-$

However, they do not provide any further explanation of the mechanism. In regard to the anode, we can watch it deteriorate as the battery discharges electricity, but we cannot see the cathode deteriorate. I performed a set of experiments and arrived at the conclusion that it is the oxygen that reacts at the cathode.

Keywords: bincho charcoal, battery cell, anode, cathode

1. Introduction

Because global warming has recently become an international concern, we need to identify new, clean energy sources. One of these clean energy sources is the bincho charcoal battery cell.

The bincho charcoal battery cell is a new type of battery that generates electrical energy with bincho charcoal. (Figure 1) Although we have determined that the battery can generate energy without emitting carbon dioxide, it is not yet ready for practical use. To improve the battery performance, it is necessary to clarify how it works.

Previous studies have researched the factors that decide the power of the battery. Akaho and Matsunaga reported the fact that as an electrode of this battery, bincho charcoal is better than any other metal. (2003[1]) Matsumura reported that the concentration of the electrolyte and the size of the bincho charcoal has a positive correlation with the power of the battery. (2007[2]) Hukunaga Paku found that the best charcoal battery used silk for the electorode.(2010[3]) Okazaki T. Yamaguchi reported that bincho charcoal is the best charcoal to use as an electrode, and they also reported that providing a moderate amount of water helps to increase the power of the battery.

Studies in the literature show the following formula:

cathode (O_2) $3O_2 + 6H_2O + 12e^- \rightarrow 12OH^-$

Anode (Al) $4Al \rightarrow 4Al^{3+} + 12e^-$

However, they do not provide any further explanation of the mechanism. In regard to the anode, we can watch it deteriorate as the battery discharges electricity. [4] However, we cannot see the cathode deteriorate. I performed several experiments, which are described below, and came to the conclusion that it is the oxygen that reacts with the cathode.

The process of making the battery cell involves rolling a sheet of paper towel that is soaked in saline (this works as an electrolyte) around the washed bincho charcoal and then rolling a sheet of aluminum foil around the paper towel. This is the bincho charcoal battery cell, and it generates electricity. This battery is good for the environment because it does not contain hazardous material. [5]

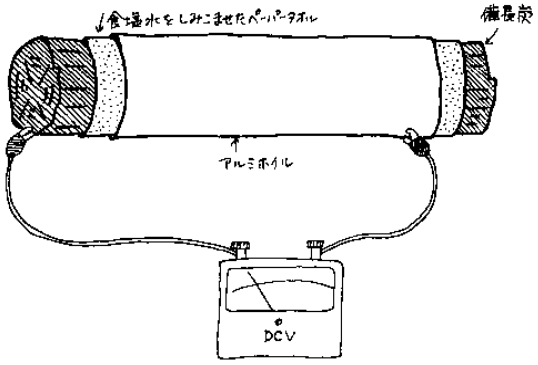


Figure 1.Model of bincho charcoal battery cells

2. Methods and Materials

In this study, I used bincho charcoal (8.5 cm long, and 2 cm in diameter), aluminum foil (6.5 cm×5 cm), paper towels (6.5 cm×15 cm), a propeller (which is used to verify that the battery cells are generating electricity), and 10% saline as the electrolyte.

To prepare for the experiment, two tasks needed to be completed.

First, I made the bincho charcoal battery cells. I rolled a sheet paper towel that was soaked in saline around the washed bincho charcoal and then rolled a sheet of aluminum foil around the paper towel. This is a bincho charcoal battery cell, and it generates electricity. Second, I built a circuit similar to that shown in figure 2. This is the end of the preparation.

I used the bincho charcoal battery cells and measured the voltage and the amount of time the propeller was spinning. After the battery discharged and the propeller stopped spinning, I removed the aluminum foil and the paper towel and washed the bincho charcoal in water as quickly as possible. After that, I rolled a sheet of paper towel that was soaked in saline around the washed bincho charcoal and rolled a sheet of aluminum foil around it quickly. I repeat this operation continuously a total of six times.

After repeating the operation six times, for the seventh step of this experiment, I left the dead battery cell out of the water after washing it and dried it in air, allowing it to charge with oxygen. Then, I rolled a sheet of paper towel that was soaked in saline around the washed bincho charcoal and rolled a sheet of aluminum foil around the paper towel. The cell was then placed into the circuit, and the voltage and the amount of time the propeller spun were measured. Using this procedure, I can verify that the battery regained power after charging with enough oxygen. (I call this operation “the seventh operation” in this study).

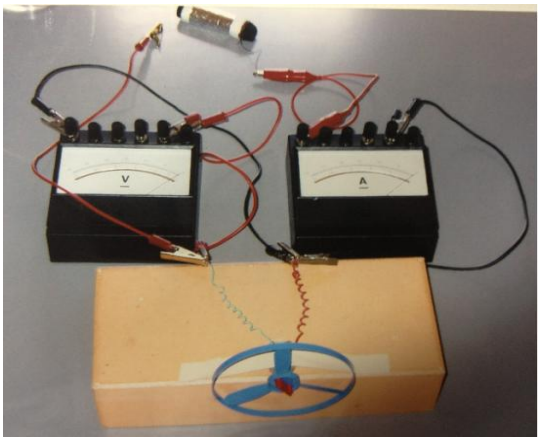


Figure 2.The circuit of this experiment

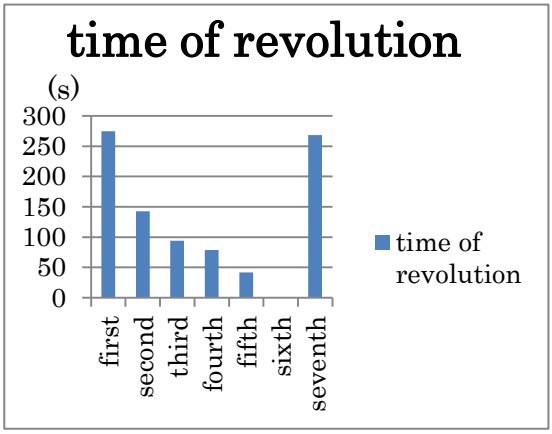
3. Results

Through the six initial operation cycles, I can examine whether bincho charcoal battery cells use the oxygen stored in them when discharging by focusing on the reduction of the power of the battery as I continuously repeat the operation.

There is a negative correlation between the number of times I continuously repeated the operation and the period that electricity was generated and between the number of times I continuously repeated the operation and the voltage of the battery.

Specifically, the amount of time the propeller would spin grew shorter and shorter and the voltage of the battery became lower and lower as I repeated the operation. After repeating the operation five times, the voltage became so low that the propeller would not spin during the sixth cycle.

I also determined that in the seventh operation (after replenishing the charcoal with enough oxygen), the battery regained the power it had in the first operation (Figure 3).



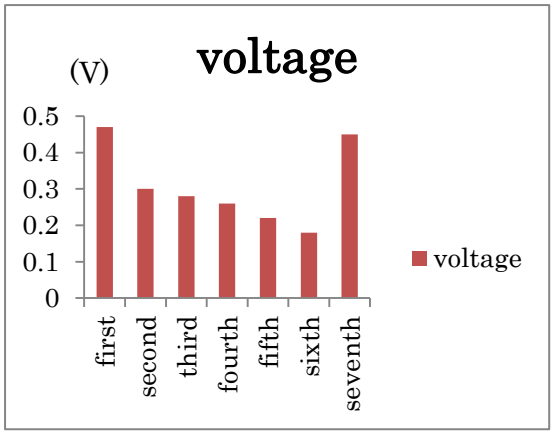


Figure 3.Reduction of the power of the battery used in our study (The ordinal numbers express how many times I repeated the same operation. The seventh operation was performed after replenishing the cell with enough oxygen.)

4. Discussion

In our study, there is a negative correlation between the number of times I continuously repeated the operation and the amount of time that electricity was generated and between the number of times I continuously repeated the operation and the voltage of the battery cell. Specifically, the amount of time the propeller spun grew shorter and shorter and the voltage of the battery became lower and lower as I repeated the operation. After repeating the operation five times, the voltage became so low that the propeller would not spin during the sixth cycle. However, in the seventh operation (after replenishing the cell with enough oxygen), the battery regained the power it had in the first operation.

Although previous studies have assumed that the anode of the bincho charcoal battery cells requires oxygen, I identified few studies that provided evidence for this assumption.

The results of our study can be interpreted as follows: because the amount of oxygen stored in the bincho charcoal decreased as I repeated the operation, the battery lost power and could spin the propeller for only a short period of time. Considering this result, I could conclude that the cathode of the bincho charcoal is oxygen.

Our study has some limitations. In the first six operation cycles, the battery cell was exposed to the least amount of air possible, but there was a moment when the battery cell was exposed to air; for example, while rolling a sheet of paper towel around the bincho charcoal or while the cell was discharging. Because of this, I can only confirm that the cathode of the bincho charcoal is oxygen from the result.

There is room for further study in an oxygen-free environment. This would allow us to explore the behavior of the cell without oxygen. (This experiment was performed at home, not in a laboratory.) Without exposure of the bincho charcoal to air, I could correctly measure the amount of oxygen a piece of bincho charcoal stores and uses for generating electricity.

5. Conclusions

An experimental study was conducted to validate the assumption that the cathode of the bincho charcoal battery cells is the oxygen stored in the bincho charcoal.

This assumption was investigated by minimizing the amount of time the bincho charcoal battery cells were exposed to air.

The experimental results indicatedthat there is a negative correlation between the number of times I continuously repeated the operation and the amount of time electricity was generated and between the number of times I continuously repeat the operation and the voltage of the battery cell.

The results obtained suggest that the cathode of the bincho charcoal battery cells is the oxygen stored in the bincho charcoal.

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Trade policies by regional integration

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Abstract: Today, many countries are conducting free trade policies based on neo-liberalism, and the integration of the world is in progress. However, it has been suggested that free trade policies cause serious problems, such as the expansion of economic disparities. On the basis of this situation, I have analyzed the trade policies of ASEAN. ASEAN is a regionally integrated association that conducts free trade policies. Therefore, an analysis of ASEAN is useful in understanding the world. To reveal the effectiveness of free trade policies, I analyzed ASEAN using two theories: the theory of comparative advantage and the World-Systems theory. Using these two theories, I scrutinized the changes in ASEAN's industrial structure through trade policies. In Japan, no studies have been conducted on ASEAN using the World-Systems theory. This article concludes that free trade policies do not always benefit countries that have adopted these policies.

Key Words: Trade policy, Free trade, The theory of comparative advantage The World-Systems theory, Regional integration.

1. Introduction

Considering the current situation of the world economy, many countries are conducting free trade policies, and the integration of the world market is underway. Although these policies expand economic disparities, can free trade really enrich people's lives?

To consider this issue, it is useful to analyze ASEAN because it has achieved economic growth through free trade policies and regional integration. In short, ASEAN combines conditions, economic integration and free trade policies that are the goals of the world economy. Therefore, by examining the impact of free trade policies on ASEAN, we can predict what type of effects may emerge in the world through the promotion of free trade policies.

In this paper, I analyzed ASEAN by using the theory of comparative advantage and the World-Systems theory. The World-Systems theory has been used in various analyses. Although this theory has been widely adopted in the analyses of European countries and societies, no previous reports have analyzed ASEAN using this theory, even if we consider CiNii articles. Therefore, I believe that analyzing ASEAN using the World-Systems theory can present a new perspective.

2. Materials & Methods

This research is based on an analysis of statistical data on ASEAN. In this paper, I analyze these data using two theories, the theory of comparative advantage and the World-Systems theory. In the following, I will briefly introduce these two theories.

First, I will explain the theory of comparative advantage. Free trade involves the elimination of those that hinder trade, such as import / export regulations and tariffs on trade. People benefit as a result because the sales of their products increase or the flow of imports and exports becomes smooth, with the inclusion of cheap goods. The main theory is that the basis of free trade is Ricardo's "theory of comparative advantage".

This theory suggests that mutual benefits produce free trade because countries produce and export goods that have a comparative advantage relative to each other. By rapidly increasing the production of goods with a comparative advantage and replacing those goods through trade, countries can obtain goods for more than just further production, improving their economic welfare. In other words, the expansion of free trade contributes to the development of the world economy because, in contrast to free trade, protectionist trade policies (e.g., tariffs and import restrictions) are not advantageous.

Table 1. Examples of comparative advantage
The amount of necessary labor per unit of production year

	United Kingdom	Portugal
Wool	100	90
Wine	120	80

Comparative costs	100/120 = 0.83	90/80 = 1.125

Table 2. specialization in the production of goods comparative advantage

	United Kingdom	Portugal
Wool	220	0
Wine	0	170
Comparative costs	220/100 = 2.2	170/80 = 2.215

The total amount of goods produced by the two countries = 2.2 + 2.215 = 4.325

As shown in Table 1, the UK requires 100 people to make one unit of wool and 120 people to make a single unit of wine, whereas Portugal requires 90 people to make a unit of wool and 80 people to make a unit of wine. The comparative costs of the two countries are 0.83 for the United Kingdom and 1.125 for Portugal. The comparative advantage of wool (in this case, it can be produced more cheaply than either woolen cloth or wine) is shown in Table 2. To specialize in the production of goods with a comparative advantage, Portugal's wine production volume increases. Trade can be replaced by an amount corresponding to the increase in production volume, so the people of both countries will be able to obtain a larger number of goods than before. Therefore, this is considered free trade.

Thus, in the theory of comparative advantage, protectionist measures, such as tariff barriers, must be denied for the state to protect domestic industries. For example, although there are protectionist trade policies for rice farmers in Japan over high tariffs on rice, the costs for the protection of agriculture in Japan are similar to or greater than the benefits to be obtained by it. For this reason, the public claims that products will be obtained cheaply, economic welfare is improved, and liberalization in agriculture should be performed by importing agricultural products from overseas.[1]

In the real world economy, one example of a country that has achieved economic growth by promoting free trade policies is Chile. As seen in Figure 3 on the rate of economic growth in Chile, the Chilean Pinochet junta was established in 1973. Chile introduced the idea of Milton Friedman's economic neoliberalism. Chile subsequently underwent a policy shift to an open economy led by the private sector based on a market fundamentalism policy from an industrial development-driven nation ahead of other Latin American countries and achieved economic growth. Then, Chile achieved sustainable growth and was on track to overcome the debt crisis of the early 1980s. Chile has been acclaimed as an "honor student" in the economy of Latin America. In the 1990s, the Chilean economy expanded, supported by growth in resource prices and exports and an average real economic growth rate in 1991-1997 of 8.3%.[2]

Next, I will introduce "The World-Systems theory", which is critical of free trade. The World-Systems theory was advocated by the American sociologist I. Wallerstein. This theory states that "Core" countries with hegemonic economies develop in a manner that contributes to the economic system of the "core" country and are classified as "peripheral" to the rest of the world. Based on this theory, under free trade, countries will produce goods with a comparative advantage in production, but they will only produce these goods. When distinguishing between countries with primary industries, such as agricultural products, and high value-added countries that produce high value-added products, the economic gap between those countries is identified. As a result, although countries that produce high value-added goods become "core" countries, countries that produce primary industry goods become "peripheral". The relationship between "core" and "peripheral" is immobilized, and "peripheral" is incorporated into the economic system of the "core" country.

An example of such a world system existed in Eastern Europe during 16th-century serfdom.–Western Europe became rich through the development of trade and commerce with other regions. As a result, in Western Europe, the population and the demand for food increased. At this time, Ricardo showed a comparative advantage in Western Europe, and they specialized in the production of industrial products, such as wool. In Eastern Europe, the countries were inferior to Western Europe in the production of industrial products and agricultural products and industrial raw materials for export to Western Europe. For industrial goods, the profit is larger than for agricultural products. Western European countries became enriched by increasing their economic strength, whereas the economic strength of countries in Eastern Europe did not increase as much, reinforcing the economic disparity between Eastern Europe and Western Europe. Thus, the world system of the "core" Western Europe and the "peripheral" Eastern Europe was formed. [3][4][5]

3. Results

3.1. ASEAN trade policy

ASEAN has the following aims:

①the activation of intra-regional trade;

②the promotion of intra-regional investment and foreign direct investment;

③strengthening the international competitiveness of local industry.

The ASEAN Free Trade Area (AFTA) and the ASEAN Preferential Trade Agreements (ASEANPTA) were introduced to achieve these aims. To expand intra-regional free trade, ASEANPTA was introduced in 1977. This is an agreement that allowed ASEAN countries to introduce policies such as the Margin Of Preference (MOP), a preferential margin implement that measures preferential application. The customs duty for which MOP is applied to the items for PTA is not applied to the items for PTA, and the importing country of ASEAN has 50% of the Most-Favored-Nation treatment (MFN) rate. In an attempt to promote the liberalization of intra-regional trade due to the removal of tariff barriers and non-tariff barriers within ASEAN and AFTA, this concept aims to strengthen the competitiveness of ASEAN and to activate the regional economy as a production base for the international market. More specifically, the ASEAN countries conduct a Common Preferential Tariff (CEPT). Thus, AFTA and ASEANPTA are policies that aimed to introduce free trade. [6][7]

However, given the situation in Japan in recent years, public opinion about participation in the Trans-Pacific Partnership (TPP), a free trade agreement, is not unified. It can be said that the question of the free trade is emerging. Therefore, I want to consider the trade policies of ASEAN by using two theories about free trade.

3.2. The current situation as seen from the statistics of ASEAN

Table 3. GDP per capita

	GDP per capita (10000$)
ASEAN	0.31
EU	3.24
NAFTA	3.8
MERCOSUR	1.06

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Worldwide, the main bodies of economic integration are ASEAN, EU, NAFTA, and MERCOSUR. ASEAN has the largest population of these, with 587,170,000 people and continuing high economic growth. However, as shown in Table 3, the GDP per capita of ASEAN is much lower than the other economic integration bodies.

Furthermore, there are large economic disparities within ASEAN. According to Table 4 below, the GDP per capita of Singapore is the highest in ASEAN, at $43,324. Among the CLMV countries, the GDP per capita of Myanmar is the lowest at $495, and Vietnam is the highest at $1,172.

Table 4. Comparison of per capita GDP of ASEAN (2010)

Country	GDP per capita($)
Singapore	43,324
Brunei	26,367
Malaysia	8,519
Thai	4,672
Indonesia	3,039
Philippines	2,132
Vietnam	1,172
Laos	1,164
Cambodia	802
Myanmar	495

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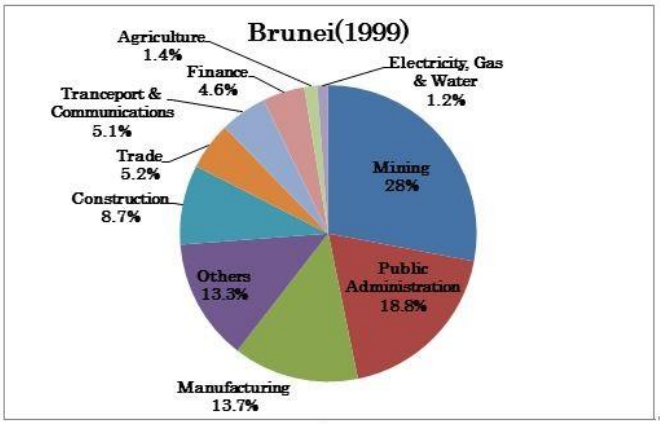
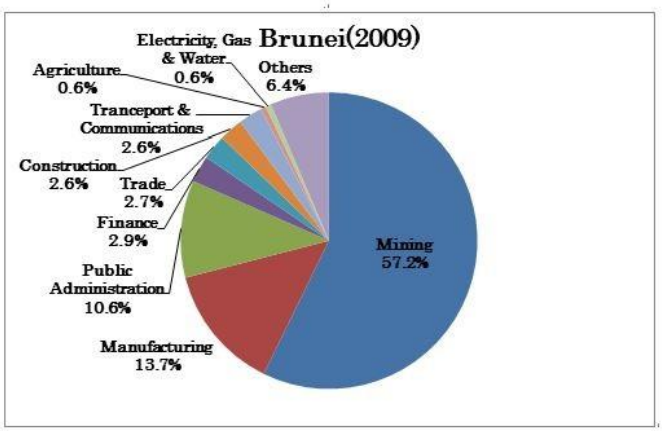
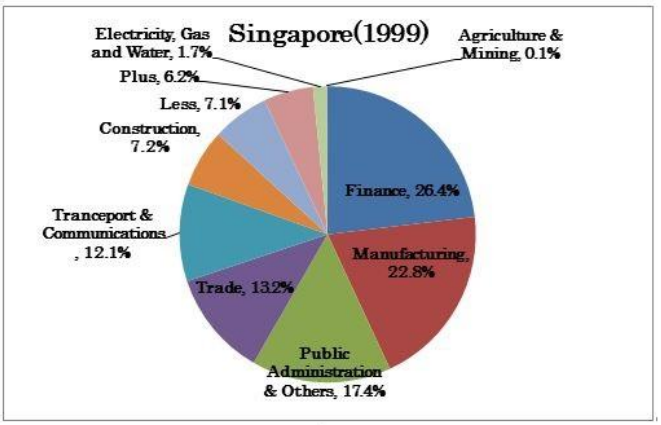
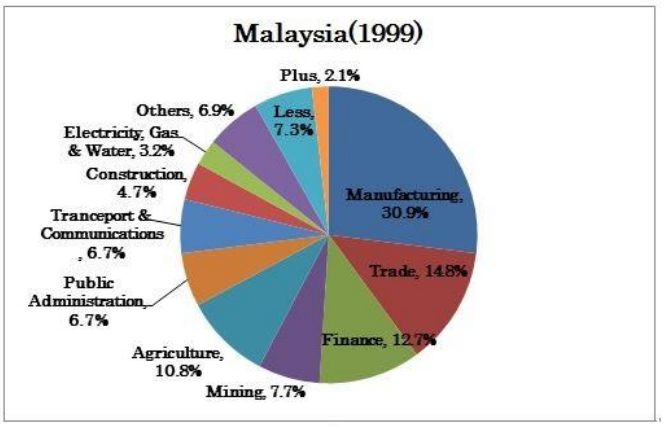
As shown in Table 5 and Table 6, the industrial structure of ASEAN countries and the comparison of

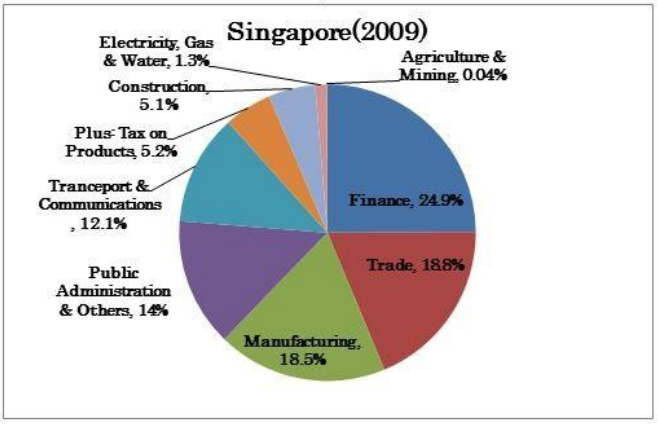
the GDP of ASEAN countries, the financial industry in Singapore is a tertiary industry that accounts for 24.9% of the largest of the gross domestic products. In contrast, the main industries of the CLMV countries are agriculture, forestry and fisheries products as well as artifacts and sewing and labor-intensive manufacturing industries, such as the shoemaking. In CLMV countries, agriculture accounts for the largest proportion of the gross domestic product. This fact suggests that CLMV countries have not been able to move away from an agricultural industry even after they become members of ASEAN. In any ASEAN country, the GDP has grown after the country joined ASEAN. However, Singapore has recorded significant growth in nominal GDP per capita. The GDP per capita of Singapore almost doubled in 1995 when the CLMV countries took part in ASEAN.

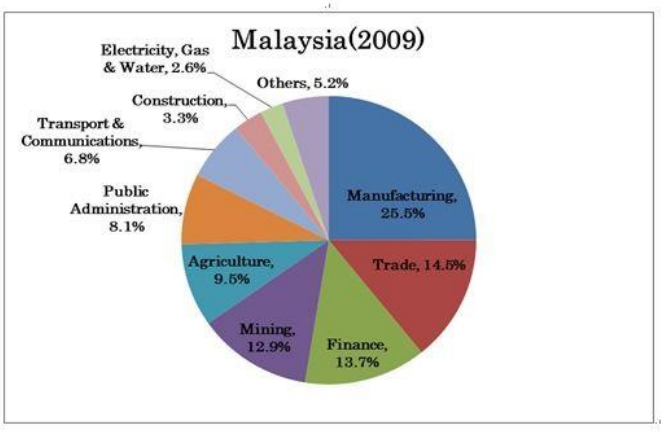


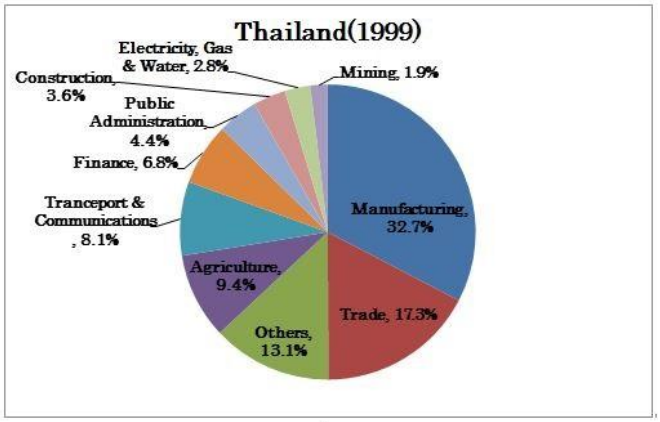


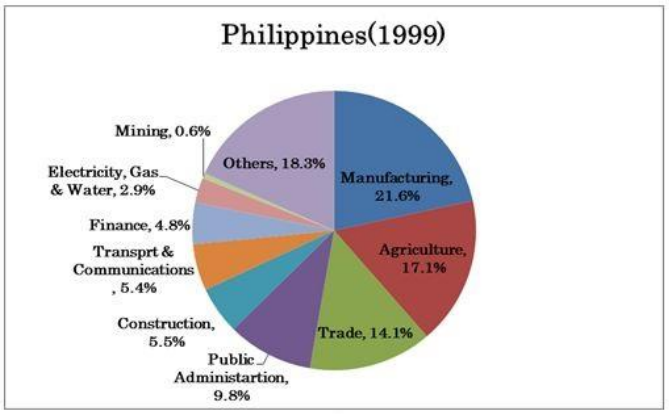


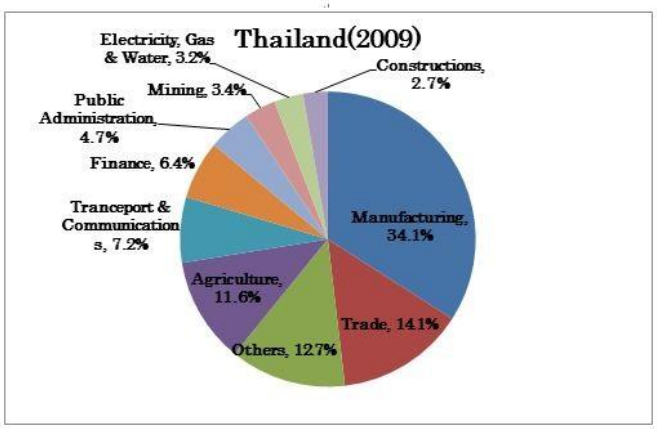




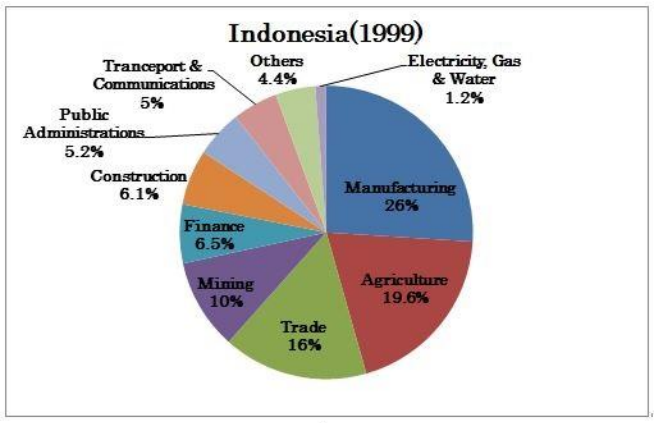


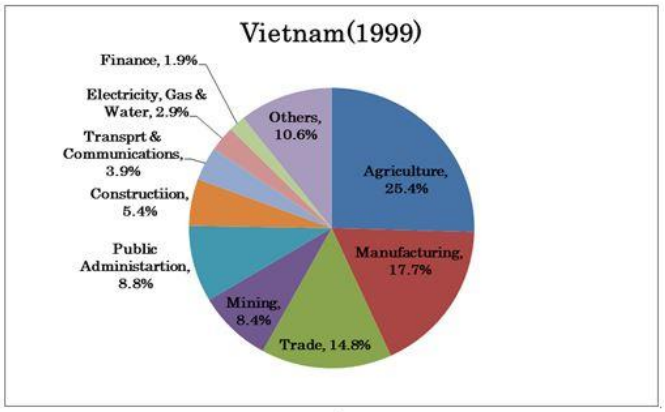

Thailand(1999)
Electricity, Gas & Water, 2.8%
Mining, 1.9%
Construction, 3.6%
Public Administration, 4.4%
Finance, 6.8%
Tranceport & Communications, 8.1%
Manufacturing, 32.7%
Agriculture, 9.4%
Trade, 17.3%
Others, 13.1%

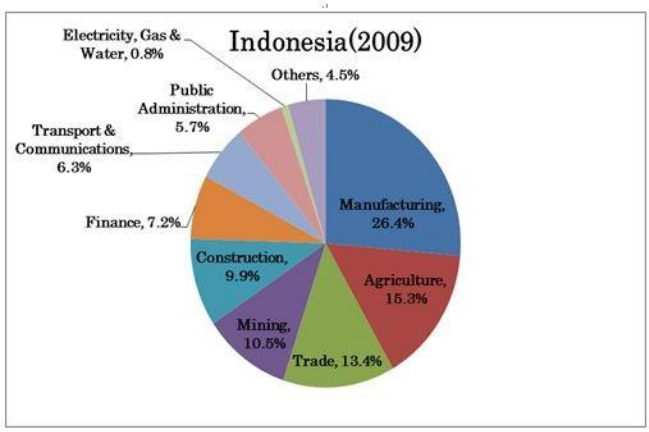

Philippines(1999)
Mining, 0.6%
Others, 18.3%
Manufacturing, 21.6%
Electricity, Gas & Water, 2.9%
Finance, 4.8%
Agriculture, 17.1%
Transprt & Communications, 5.4%
Construction, 5.5%
Trade, 14.1%
Public Administartion, 9.8%

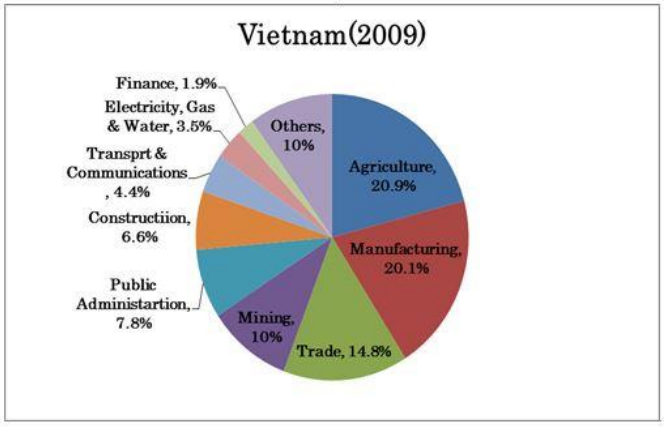

Thailand(2009)
Electricity, Gas & Water, 3.2%
Constructions, 2.7%
Mining, 3.4%
Public Administration, 4.7%
Finance, 6.4%
Tranceport & Communication s, 7.2%
Manufacturing, 34.1%
Agriculture, 11.6%
Trade, 14.1%
Others, 12.7%

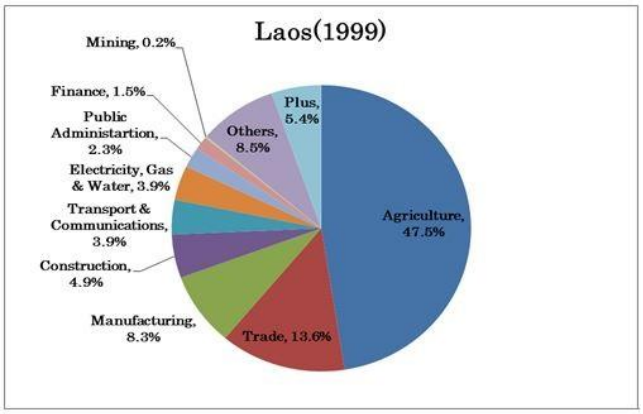

Philippines(2009)
Mining, 1.6%
Others, 20.5%
Manufacturing, 20.4%
Electricity, Gas & Water, 3.2%
Agriculture, 14.8%
Construction, 5.1%
Finance, 5.8%
Trade, 14.5%
Transport & Communications, 6.7%
Public Administration, 7.5%

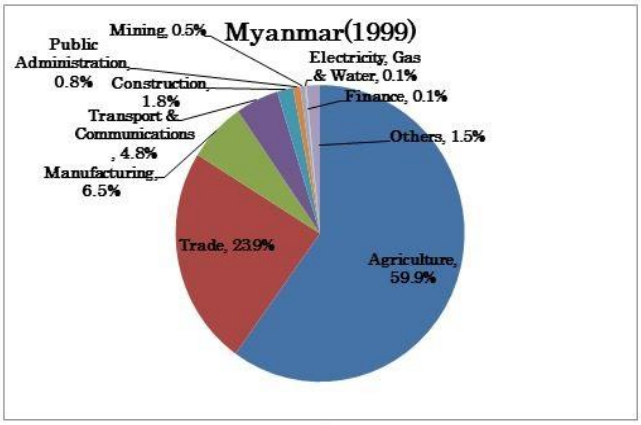

Indonesia(1999)
Tranceport & Communications 5%
Others 4.4%
Electricity, Gas & Water 1.2%
Public Administrations 5.2%
Construction 6.1%
Manufacturing 26%
Finance 6.5%
Agriculture 19.6%
Mining 10%
Trade 16%

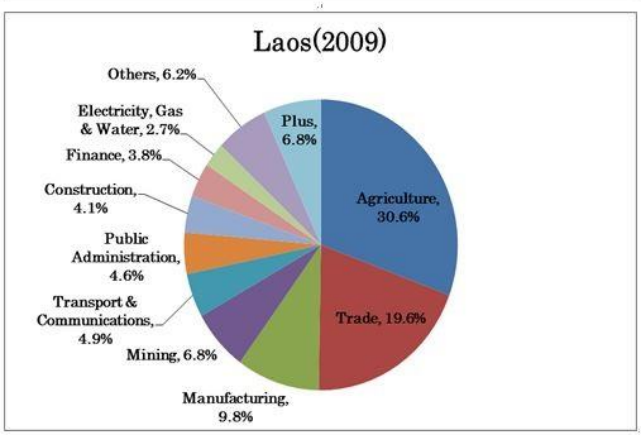

Vietnam(1999)
Finance, 1.9%
Electricity, Gas & Water, 2.9%
Others, 10.6%
Transprt & Communications, 3.9%
Agriculture, 25.4%
Constructiion, 5.4%
Public Administartion, 8.8%
Manufacturing, 17.7%
Mining, 8.4%
Trade, 14.8%

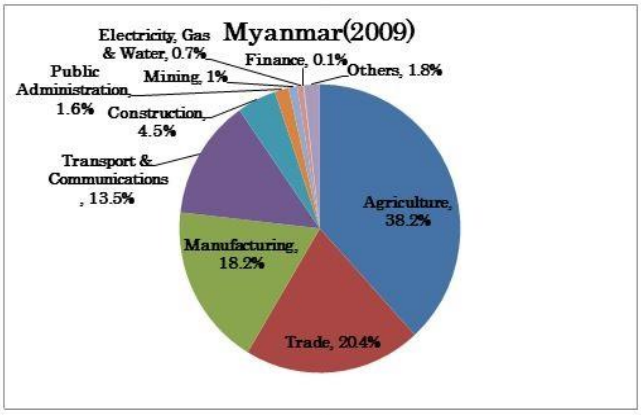

Indonesia(2009)
Electricity, Gas & Water, 0.8%
Others, 4.5%
Public Administration, 5.7%
Transport & Communications, 6.3%
Manufacturing, 26.4%
Finance, 7.2%
Construction, 9.9%
Agriculture, 15.3%
Mining, 10.5%
Trade, 13.4%

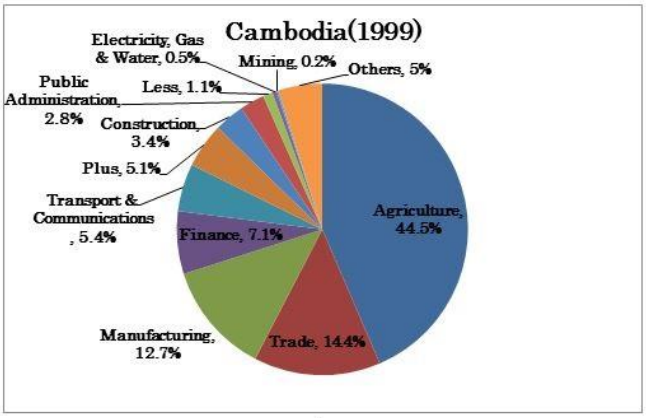

Vietnam(2009)
Finance, 1.9%
Electricity, Gas & Water, 3.5%
Others, 10%
Transprt & Communications, 4.4%
Agriculture, 20.9%
Constructiion, 6.6%
Manufacturing, 20.1%
Public Administartion, 7.8%
Mining, 10%
Trade, 14.8%











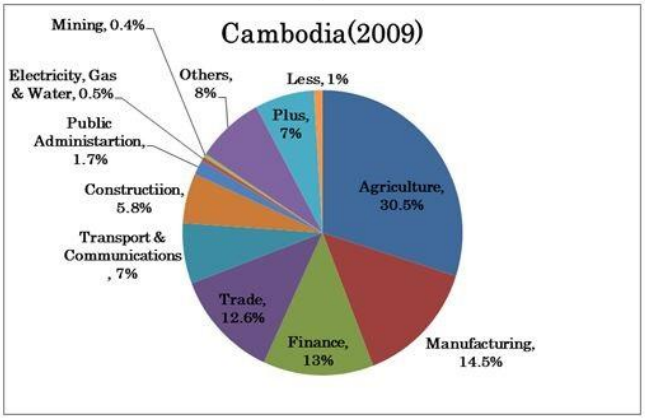


Figure1. Changes in the industrial structure of ASEAN countries
日本アセアンセンター「ASEAN －日本統計集 2011」
http://www.asean.or.jp/ja/asean/know/statistics/Latest_Stats_All(2012/7/30)

Table 5. Change in GDP per capita of ASEAN countries

Million US$

	1980	1990	1995	2000	2006	2007	2008	2009	2010
ASEAN	235, 772	357, 748	688, 611	608, 744	1, 093, 767	1, 309, 362	1,522, 195	1, 502, 608	1, 865, 372
Singapore	12, 046	38, 835	87, 062	94, 308	145, 332	177, 329	189, 384	183, 332	222, 699
Brunei	_	3, 520	4, 734	6, 001	11, 471	12, 248	14, 417	10, 733	12, 371
Malaysia	24, 938	44, 025	90, 173	93, 789	157, 050	187, 005	222, 741	193, 026	237, 959
Thailand	32, 353	85, 640	168, 019	122, 725	207, 089	246, 977	272, 578	263, 711	318, 908
Indonesia	95, 375	125, 722	223, 361	165, 521	364, 350	432, 232	511, 213	538, 457	706, 752
Philippines	35, 954	48, 932	83, 678	81, 026	122, 211	149, 360	173, 603	168, 485	199, 591
Vietnam	27, 847	6, 472	20, 798	31, 176	60, 933	71, 112	90, 302	93, 169	103, 574
Laos	1, 004	915	1, 880	1, 640	3, 564	4, 226	5, 313	5, 598	6, 461
Cambodia	_	899	3, 419	3, 653	7, 264	8, 691	11, 277	10, 871	11, 629
Myanmar	6, 255	2, 788	5, 487	8, 905	14, 503	20, 182	31, 367	35, 226	45, 428

日本アセアンセンター「ASEAN －日本統計集 2011」

4. Discussion

As shown in Table 5, which shows the industrial structure of ASEAN countries, the financial industry in Singapore includes tertiary industries and accounts for 24.9% of the GDP. This percentage is the largest in the industry that makes up the GDP of Singapore. Singapore has developed into the fifth largest financial center, after New York, London, Tokyo and Hong Kong. In contrast, in the CLMV countries, agriculture accounts for the largest proportion of the GDP. Therefore, it can be said that these countries have not been able to move away from agriculture even after participating in ASEAN. In Singapore, it is difficult to secure land for agriculture, so agriculture has not been developed in Singapore. Therefore, Singapore relies on imports for most of its groceries. In other words, Singapore is said to be in a situation of comparative disadvantage in the primary sector, including agriculture, compared with other ASEAN countries. Therefore, the theory of comparative advantage is applicable, and tertiary industries, such as the financial service industry, which is not dependent on the total land area, are developed.

The economy of Singapore has grown as the development of tertiary industries has increased the population through the influx of immigrants from abroad. CLMV countries specialize in the production of agricultural products for which they have a comparative advantage to export to Singapore due to the growing demand for food. As a result, economic disparity arose, similar to that shown in Table 5. In other words, one can think of the world system's "core" as Singapore, with the "peripheral" countries within ASEAN. CLMV is the way they are formed. Accord-

ing to Table 5 and Table 6, in any ASEAN country, the GDP has grown after the country joined ASEAN.

However, Singapore has recorded significant growth in the nominal GDP per capita, which almost doubled in 1995 when the CLMV countries participated in ASEAN. It can be said that the world system formed a "core" of Singapore between 1995 and 2000, after the accession of ASEAN CLMV countries.

Thus, as a result of promoting free trade, ASEAN was able to achieve economic growth. In contrast, as a result of the theory of Ricardo's comparative advantage, CLMV countries that specialize in the production of primary industry goods have been incorporated into the economic system of Singapore, which has specialized in tertiary industries. Large economic disparities between Singapore and CLMV countries occur, and the world system with "peripheral" countries (CLMV) and the "core" of Singapore is formed.

5. Conclusions

From a historical perspective, ASEAN, which emerged as an anti-communist military alliance and changed the mechanisms of political and economic cooperation in Southeast Asia, was established by capitalist countries that promote free trade. ASEAN promotes free trade because it was based on free trade policies, and it is conceivable that this trend will continue. Currently, large economic disparities are present within ASEAN, and ASEAN has recognized measures, such as a tariff reduction grace period for CLMV countries, to address the weak economy. This does not change the fact that ASEAN is oriented toward free trade.

However, as noted in this paper, if there is a negative impact of free trade results on comparative advantage and the spread of economic disparity and the idea of the World-Systems theory by Wallerstein is applicable, it can be suggested that free trade is not the best policy. Thus, ASEAN may require a flexible policy and must recognize that such protection is dependent on the status of international trade. They must review the policy of free trade.

In the current state of ASEAN and its continuation of the present policy of free trade around the world, only some countries will benefit, whereas other countries will face impoverishment. Therefore, the current trend toward promoting excessive free trade must be modified.

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The Culture of British Horse Racing

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Abstract: What do you think when you hear the phrase "British horse racing"? Some people think that it is only for gambling. However, some people think that horse racing is part of the culture in Britain. The purpose of this study was to examine British horse racing and to consider the features of British culture through this evaluation of British horse racing. This study is based on fieldwork, questionnaires conducted with people living in the UK, and research documents about British horse racing. The findings indicate that British horse racing is reflected in British society and is part of British culture. Most of the culture of British horse racing is related to "aristocratic culture" and the upper class, but this aristocratic culture is becoming "popular culture".

Key Word: British Horse Racing

1. Introduction

When people think about horse racing, they may initially think of gambling. Of course, some people think that horse racing is a sport or even a culture, but these concepts are rare. The first impression of horse racing is often gambling. However, the reality of British horse racing is completely different; it is related to gambling, but it also involves other aspects. This study is an effort to define British horse racing through field work and questionnaires. This section provides background knowledge on British horse racing, reviews previous studies, and proposes the research question for this study.

This section first provides the history of British horse racing, which is an important part of the background knowledge of British horse racing. Signs of British horse racing were already apparent in the first century. The ancient Celts who lived in Newmarket engraved the figure of a horse on the coins they used. Since the first century, people in Newmarket, which is one of the largest industrial horse centers in the United Kingdom, have loved horses. From the first century to the fourth century, England was controlled by the Roman Empire, but it is clear that British people began training and protecting horses during this time because the ruins of a stable for race horses that was built in the third century have been found in Yorkshire.

After the collapse of the Roman Empire, British people became more enthusiastic about horses and horse racing. Alfred the Great, who was a famous king in the ninth century, had a horse trainer who served him exclusively, and in the tenth century, Hugues Capet, the founder of the Capetiens in France, presented a horse to propose to the daughter of the British king. Thus, even in the tenth century, British people's love of horses was well known throughout Europe. Furthermore, a proclamation by the British king at that time supported this evidence; the British king forbade people from sending horses abroad for any purpose without his permission.

In the Middle Ages, the British kings' enthusiasm for horses promoted crossbreeding. Many kings desired marvelous horses, so they imported horses from foreign countries. This may be how thoroughbred horses were born in the eighteenth century. Many famous kings and queens loved horses and horse racing. Edward I, Edward III, Henry VII, Henry VIII, and Elizabeth I were all lovers of horses and horse racing. In particular, Elizabeth I is famous as a horse enthusiast. She attended many horse races sponsored by famous noblemen or noble women, was an owner of horses, had personal jockeys, and could even mount a horse.

The turning point in British horse racing was the eighteenth century. First, we can note the establishment of the Jockey Club, which unified British horse racing. This led to the birth of modern horse racing, and foreign countries' horse races largely follow the British model of horse racing developed at this time. Second, classic races were started, and the three major races originated from these races. Third, thoroughbred horses were produced. Three of the most famous types of thoroughbred horses, Byerley Turk, Darley Arabian, and Godolplin Arabian, were produced as the result of improvements in breeding. The present style of British horse racing succeeded the style formed in the eighteenth century.

This section also discusses previous studies of horse racing. Some previous researchers have studied British horse racing, such as James Rich, Theodore Cook and C. M. Prior, but these studies provided

only the history of horse racing. Even when they noted the cultural aspects, these were not the main theme of the study. These studies were presented in the history part of this section. Some researchers have discussed the cultural aspects of British horse racing, but they have addressed this issue only generally, either by explaining British culture based on the Jockey Club or the situation of classic racing, and previous studies have not included field work.

The purpose of this study is to examine British horse racing and British culture. Therefore, this study is based on fieldwork and questionnaires conducted with British people. Given that few researchers have examined the culture of British horse racing based on field work, this study is useful. However, we must refer to previous studies on the history of horse racing because history is an important factor to explain culture.

2. Methods

2.1 Field work

The researcher went to the United Kingdom and observed British horse racing in Doncaster. The race in Doncaster is one of the three major title races. The researcher went to the race with a horse trainer so he could enter the special room for celebrities. He observed the actual condition of the turf and of British horse racing.

2.2 Questionnaire

The participants were asked four questions.

1. What is your image of horse racing? Why do you have this image?
2. In Japan, many people think that horse racing is just gambling. What do you think about this?
3. For you, is British horse racing part of British culture?
   If yes, why do you think it is cultural? If no, what does British horse racing mean to you?
4. Some people say that British horse racing reflects British hierarchical society. What do you think about this opinion?

Fifty people answered the questionnaire. The participants included 15 horse trainers, 15 businessmen and 20 students.

For the first question, the researcher categorized the participants' answers into three categories: positive, negative, and neutral. If the answer included negative words, such as bad, wrong or poor, this answer was categorized as negative. Positive and neutral were categorized similarly. This approach was used to make this study easier to understand and to generalize.

3. Results

3.1 Field work

The researcher observed many features of the British turf when he attended the Doncaster race.

First, almost everyone on the turf wore suits or dresses, and some women wore traditional hats. If people wore jeans or casual clothing, they were not allowed to enter some spaces. This is a rule in British horse racing. Especially at the Royal Ascot race in June, which is sponsored by the Royal Family, there is a dress code that requires men to wear a morning coat, a long-sleeved white shirt, a necktie, and a silk hat and requires women to wear a formal dress and a hat. Second, there were varied age groups, ranging from children of six or seven years old to people eighty or ninety years old. People under eighteen years old were not allowed to gamble, but some children accompanied their parents. Third, some special rooms were arranged for celebrities. Ordinary people usually could not enter these rooms, and food and drink were served in these rooms. Fourth, some celebrities attended the race, such as famous actors or actresses or famous golf players. Fifth, the people there treat some horse trainers and jockeys as celebrities, sometimes asking for their autographs.

3.2 Questionnaire

(1) First question

There were three responses to this question: a positive response, a negative response, and a neutral response. A positive response meant that the participant thought that British horse racing was good. A negative response meant that the participant thought that British horse racing was not good. A neutral response meant that the participant thought that British horse racing had both a good side and a bad side.

Among the horse trainers, twelve out of fifteen provided a positive response, and three trainers provided a neutral response. Among the businessmen, five businessmen provided a positive response, four businessmen provided a negative response, and six businessmen provided a neutral response. Among the students, seven students provided a positive response, eight students provided a negative response, and five students provided a neutral response. In figure 1, the results are shown.

Figure 1. The responses to the first question

	Negative	Positive	Neutral
Trainers	0(0%)	12(80%)	3(20%)
Businessmen	5(33%)	4(27%)	6(40%)
Students	7(35%)	8(40%)	5(25%)

(2) Second question

There were two types of answers. The first was

that the participants thought that horse racing had aspects of gambling but was also important as a sport or culture. The second was that the participants thought that horse racing was not completely gambling but was mostly gambling. All of the trainers provided the former answer. In the case of the businessmen, 13 businessmen provided the former answer, and 2 businessmen provided the latter answer. In the case of students, 12 students provided the former answer, and 8 students provided the latter answer. In figure 2, the results are shown.

Figure 2. The responses to the second question

	The former	The latter
Trainers	15(100%)	0(0%)
Businessmen	13(87%)	2(13%)
Students	12(60%)	8(40%)

(3) Third question

There were two types of answers. The first was that British horse racing is part of British culture. The second was that horse racing is only a sport, not part of the culture.

In the case of trainers, 13 trainers thought that British horse racing was cultural, and 2 trainers thought it was a sport. In the case of businessmen, 10 businessmen thought that it was cultural, and 5 businessmen thought it was a sport. In the case of students, 12 students thought that it was cultural, and 8 students thought that it was a sport. In figure 3, the results are shown.

Figure 3. The responses to the third question

	Culture	Sport
Trainers	13(87%)	2(13%)
Businessmen	10(67%)	5(33%)
Students	12(60%)	8(40%)

(4) Fourth question

There were two types of answers. The first was that participants thought that British horse racing clearly reflected the hierarchical society. The second was that they thought that horse racing once reflected a hierarchical society, but now all groups could enjoy horse racing.

In the case of horse trainers, 8 horse trainers provided the former answer, and 7 horse trainers provided the latter answer. In the case of businessmen, 9 businessmen provided the former answer, and 6 businessmen provided the latter answer. In the case of students, 14 students provided the former answer, and 6 students provided the latter answer. In figure 4, the results are shown.

Figure 4. The responses to the fourth question

	The former	The latter
Trainers	8(53%)	7(47%)
Businessmen	9(60%)	6(40%)
Students	14(70%)	6(30%)

4. Discussion

According to the fieldwork, the British turf clearly reflects British hierarchical society. The strict dress code and the special room for celebrities are symbols of a hierarchical society. In addition, from a historical point of view, it is clear that British horse racing is a sport and pastime for upper-class people.

However, according to the results of the fourth question, some people think that British horse racing does not reflect a hierarchical society. Of course, more people can enjoy horse racing than ever before. There are still special rooms and a dress code, but some people do not think that these factors are features of a hierarchical society. This may reflect one British definition of a hierarchical society, but, in general, these factors indicate a hierarchical society.

According to the results of the questionnaire, trainers tend to think that British horse racing is not only gambling and is also cultural, whereas businessmen who are not directly involved in horse racing and, especially, students tend to think that British horse racing is mostly gambling and sport, not culture. This finding indicates that British horse racing is a culture that is shared by limited people and has certain exclusive aspects. According to the field work, people who often attend horse races tend to be upper-class people. Most of them wear luxurious clothing and carry expensive hand bags. In contrast, people who do not attend horse racing often and only know of it through the media may focus on the gambling aspects.

However, in this study, only 50 people answered the questionnaire, so the results are not necessarily generalizable. Thus, it is necessary to reexamine this study.

5. Conclusions

In conclusion, British horse racing reflects British

society, and the British turf is an example of British society. On the turf, there are many rooms, and people go into the rooms appropriate to their class.

British horse racing involves interesting contradictions. Although British people modernized horse racing and provided the model for other countries' horse racing, British horse racing now reflects an outdated society. Modernization and aristocratic culture coexist in horse racing in way that only occurs in the United Kingdom.

In a sense, this may be the antithesis of modern civilization with its excessive expansion of freedom. This study can provide clues to improve modern society.

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The Antibacterial Effect of Tea

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Abstract: Previous studies have shown that the components of tea have favorable effects. However, little is known regarding the differences in effects among different types of tea leaves and blended tea, which is made by mixing several types of tea. Here, we examined the antibacterial action of five types of tea (green, oolong, black, Pu'er, and blended) by observing bacterial growth on agar media containing tea. We first demonstrated that green and oolong tea were effective in inhibiting the growth of bacteria, whereas black tea was not effective, and Pu'er tea was poorly effective. This result suggests that the oxidative fermentation of tea leaves makes tea ineffective. We also found that blended tea had superior effects although it consisted of four types of tea, which suggests that the tea ingredients, such as green and oolong tea, work independently.

Key Words: antibacterial effect, tea, blended tea, bacteria

1. Introduction

Tea is a widely consumed beverage all around the world. Most Japanese people drink green tea every day. There is an afternoon tea custom, and people drink black tea in England and many other countries. Thus, tea plays an important role in our daily life. The effects of tea have been reported, such as reducing the risk of coronary artery disease (Sano et al., 2004 [1]), heart disease (Vinson, 2000 [2]) and cancer (Yang et al., 2011 [3], Tang et al., 2009 [4]). Previous studies on tea have shown that it has some effective components, such as polyphenols ([3]) and catkins (Hara et al., 1989 [5], Hara & Tono-oka, 1990 [6]).

Some previous studies have compared the effects of green and black tea [4] or these two types of tea and oolong tea [2], but little is known about other teas, such as Pu'er tea. Additionally, the effect of blended tea has not yet been studied. In this study, the effects of green, oolong, black, Pu'er, and blended tea were compared. An experiment to observe bacterial growth was conducted.

This study had two aims. One was to determine which type of tea is most effective at inhibiting bacterial growth, and the other was to determine the antibacterial effect of blended tea.

A hypothesis was proposed before the experiment. First, green tea was expected to be the most effective at inhibiting the growth of bacteria because many products contain green tea elements. Second, blended tea was predicted to show average effectiveness because it consisted of four different types of tea.

2. Materials and Methods

2.1. Preparations

(1) Tea

In this study, four types of tea leaves were used; green, oolong, black, and Pu'er tea. All of the tea leaves used in this research were purchased from an online store ('Ayaka-en' in Shizuoka, Japan). The difference between the tea leaves is due to the oxidative fermentation of leaves (Graham, 1992 [7], Cabrera, 2006 [8]). The level of oxidation is as follows: green < oolong < black << Pu'er. Green tea leaves were not oxidized or fermented. Oolong tea leaves were semi-oxidized and not fermented. Black tea leaves were perfectly oxidized but not fermented. Pu'er tea leaves were fermented with microorganisms but not oxidized. Original blended tea leaves were obtained by mixing the same amounts of the four types of tea leaves by the researchers.

The five types of tea (green, oolong, black, Pu'er, and blended) were prepared in the same manner. Briefly, 12.5 g of each type of tea leaf was extracted for three minutes in 100 ml of boiled water.

(2) Bacteria

The bacteria used in this research were collected from the researchers (the oral cavity, hands and feet). Eight species of bacteria were chosen according to their diagnostic characteristics (color, size, and shape) and incubated separately at 37 degrees for one day.

(3) Agar Medium

Agar culture media were supplemented with tea in 20 ml petri dishes. The agar medium was previously prepared and solidified. It was used after it was melted in a water bath. In total, 21 different types of media were prepared (five types of tea (green, oolong, black, Pu'er, and blended) and four tea concentrations (1%, 4%, 10%, and 25% tea extracts by volume) in the medium and the control (no tea in the me-

dium)). Usually, the concentration of tea consumed is 10% (usual); 25% (strong) is stronger than usual, and 1% (weakest) and 4% (weaker) are weaker than usual. First, tea extracts, agar medium, and Petri dishes were prepared. Then, tea and medium were added to the Petri dish. Four species of bacteria were observed in each dish. To examine eight species of bacteria, two sets of the 21 different types of media were prepared.

2.2. Procedure

This experiment was conducted in the following manner. First, incubated bacteria were inoculated onto agar media that contained tea. To spread bacteria equally, four lines (same length and width) were drawn on the back sides of dishes, and bacteria were inoculated along each line by the inoculating loop. Next, the plates were put in an incubator at 37 degrees for one day. Then, the bacterial growth was examined by eye. Finally, each condition was compared.

3. Results

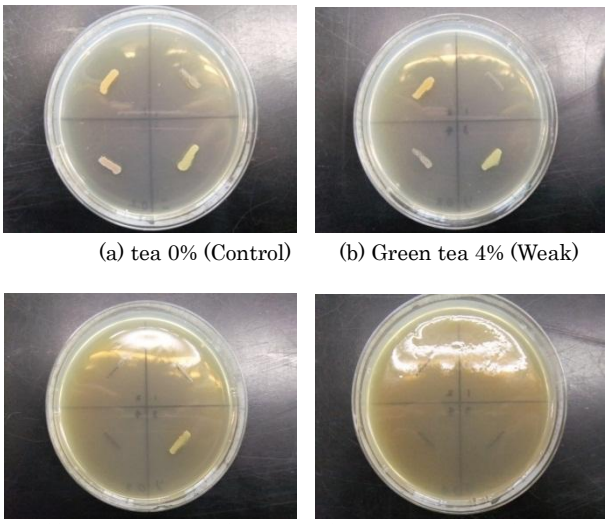
3.1. Concentration

Figure 1 shows representative images of bacterial growth, in which lines indicative of bacterial colonies can be observed. In the control medium (Figure 1 picture (a), tea 0%), all species of bacteria grew. In the weak medium (picture (b), green tea 4%), some colonies became smaller, but growth was not completely inhibited. In the usual medium (picture (c), green tea 10%), three out of four colonies were completely inhibited. In the strong medium (picture (d), green tea 25%), all bacteria showed completely inhibited growth. Similar results were obtained for other bacteria.

3.2. Types of Tea

Table 1 shows the type and concentration of each tea that inhibited the growth of each bacterium. The column shows the concentration of tea (by volume) in each medium. The row shows the type of tea in the medium. The circles in each box indicate that the tea was able to completely inhibit bacterial growth; in other words, there was no colony observed on the line. In contrast, the crosses indicate that the tea was not able to completely inhibit bacterial growth, and small colonies were observed.

The results of Table 1 were combined into Figure 2. In this graph, the height of the bars represents the number of bacterial species that were inhibited by each type of tea.



(a) tea 0% (Control) (b) Green tea 4% (Weak)

(c) Green tea 10% (Usual) (d) Green tea 25% (Strong)

Figure 1. The growth of bacteria on the media

3.3 Summary of Results

The higher the concentration of tea, the fewer bacteria grew. In the medium that contained 25% green, oolong, black, or blended tea, all species of the bacteria showed growth inhibition. Green and oolong tea were effective even when the concentration of tea was weak. Black tea showed strong inhibition at usual strength and stronger. Pu'er tea only had an effect at the high concentration, but it did not inhibit the growth of three out of eight species of bacteria. Blended tea performed similarly to green and oolong tea. However, 1% blended tea inhibited the growth of Bacteria 2, although they grew in the green and oolong tea medium.

4. Discussion

4.1 Analysis of Results

As reported in previous studies, tea had an antibacterial effect (Okubo et al., 1991 [9]). Consistent with the results of Okubo et al. (1991) [9], a higher concentration of tea in the agar medium resulted in less bacterial growth.

Green, oolong and blended tea showed similar results. One of the two differences in results among these three types of tea is that 10% oolong and black tea inhibited the growth of Bacteria 3, whereas green tea did not. The other difference is that 1% blended tea prevented Bacteria 2 from growing. These differences can be regarded as an error. In this study, we determined whether tea could completely inhibit bacterial growth. Even when the tea inhibited almost all of the bacterial growth but a small colony remained, that case was regarded as failure to completely inhibit growth. We found that 10% green tea inhibited almost all of the growth of Bacteria 3, and 1% green and oolong tea inhibited almost all of the growth of Bacteria 2. Therefore, the effects of

Table 1. Results of bacterial growth by species

Bacteria 1

Tea	1% Weakest	4% Weak	10% Usual	25% Strong
Green	○	○	○	○
Oolong	○	○	○	○
Black	×	○	○	○
Pu'er	×	×	×	○
Blended	○	○	○	○

Bacteria 2

Tea	1% Weakest	4% Weak	10% Usual	25% Strong
Green	×	○	○	○
Oolong	×	○	○	○
Black	×	×	○	○
Pu'er	×	×	×	○
Blended	○	○	○	○

Bacteria 3

Tea	1% Weakest	4% Weak	10% Usual	25% Strong
Green	×	×	○	○
Oolong	×	×	○	○
Black	×	×	×	○
Pu'er	×	×	×	×
Blended	×	×	×	○

Bacteria 4

Tea	1% Weakest	4% Weak	10% Usual	25% Strong
Green	×	○	○	○
Oolong	×	○	○	○
Black	×	×	○	○
Pu'er	×	×	×	○
Blended	×	○	○	○

Bacteria 5

Tea	1% Weakest	4% Weak	10% Usual	25% Strong
Green	×	○	○	○
Oolong	×	○	○	○
Black	×	×	○	○
Pu'er	×	×	×	○
Blended	×	○	○	○

Bacteria 6

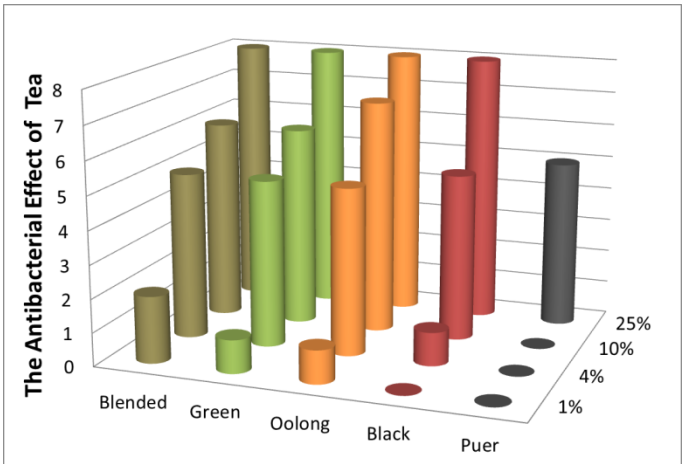
Tea	1% Weakest	4% Weak	10% Usual	25% Strong
Green	×	○	○	○
Oolong	×	○	○	○
Black	×	×	○	○
Pu'er	×	×	×	○
Blended	×	○	○	○

Bacteria 7

Tea	1% Weakest	4% Weak	10% Usual	25% Strong
Green	×	×	×	○
Oolong	×	×	○	○
Black	×	×	×	○
Pu'er	×	×	×	×
Blended	×	×	○	○

Bacteria 8

Tea	1% Weakest	4% Weak	10% Usual	25% Strong
Green	×	×	×	○
Oolong	×	×	×	○
Black	×	×	×	○
Pu'er	×	×	×	×
Blended	×	×	×	○



	Blended	Green	Oolong	Black	Puer
1%	2	1	1	0	0
4%	5	5	5	1	0
10%	6	6	7	5	0
25%	8	8	8	8	5

Figure 2. The antibacterial effect of tea

green, oolong, and blended tea were thought to be approximately equal.

Black tea was weaker than the three types of tea above. Pu'er tea was weakly effective, except at the highest concentration of 25%.

4.2 Evaluating the Hypothesis

As expected before the experiment, green tea had strong antibacterial effects, but oolong and blended tea had approximately the same strength as or were stronger than green tea.

Blended tea had a much stronger effect than predicted. The reason may be that blended tea consists of all other types of tea, and the ingredients contained in green and oolong tea worked well.

4.3 Implications

Black tea had a weaker effect than green, oolong, and blended tea. Pu'er tea had the weakest effect. These results suggest that the oxidative fermentation of tea makes tea ineffective in inhibiting the growth of bacteria. According to Cabrera et al. (2006) [8], all teas contain similar amounts of flavonoids, but their chemical structures are different. Green tea contains more catechins (simple flavonoids), while the oxidation of the leaves used to make black tea converts these simple flavonoids into theaflavins and thearubigins. Moreover, fermentation with microorganisms makes tea much weaker. Thus, catechins or other ingredients that change when tea is oxidized or fermented with microorganisms are considered to be relevant to inhibit the growth of bacteria.

4.4 Limitations of this Study and Future Considerations

In this study, the antibacterial effect was evaluated by observing the colony by eye, and the results were based on whether tea completely inhibited the growth of bacteria. Therefore, the results could not be compared or discussed in detail. In the future, other methods that can evaluate the amount of bacterial growth or inhibition are needed.

5. Conclusions

In conclusion, from the results of the experiment, green, oolong, and blended tea are the most effective at inhibiting the growth of bacteria. The effects of blended tea were much stronger than expected. It was suggested that the oxidative fermentation of tea leaves makes tea ineffective in inhibiting bacterial growth.

Green, oolong, and blended tea elements can be used in some products or medicine. For example, because offensive odors can be caused by bacterial growth and one species of the bacteria we used was collected from our feet, foot deodorant products using an insole with effective tea elements could be proposed. Moreover, medical uses of the antibacterial effect of tea, such as influenza prophylaxis by gargling with black tea, have already been researched and proved to be effective (Iwata et al., 1997 [10]).

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How We Decide Whether to Eat an Animal as Meat

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Abstract: A previous study suggests that meat nutrition is one criterion to determine whether it is good to eat. However, little is known about the criteria that result in not eating a particular meat source. In this study, we calculated a positive correlation coefficient between mammal weight and their annual global meat production and a weakly negative correlation between mammal carnivorousness and meat production. This result indicates that there is no relationship between carnivorousness and the likelihood of being eaten but that bigger animals are more likely to be eaten.

Key words: Carnivorousness, Size, Without analyzing

1. Introduction

There are approximately 180 types of mammals in the world [2], but only 10 or so are considered food sources.

We are accustomed to this, and we call it food culture. However, little is known about the reason we have chosen these animals to eat.

Kamiya (2002)[1] noted that dog meat is popular in Korea and that dog meat has much less cholesterol than beef or pork. It is also known that sheep meat has high iron and vitamin B2 content, which prevents our body from aging[9][10]. These factors suggest that healthy meats are likely to be eaten.

However, this health aspect is not an intuitive criterion. For example, a chemical analysis is needed to understand the nutrient properties of certain meats, but the procedure is very costly and difficult to perform.

Therefore, new intuitive, low-cost criteria are desirable. If new intuitive criteria are identified, they may be useful for advancing both the food industry and culture because they may identify meat sources not previously considered.

In this paper, we focus on animal size and carnivorousness. To conduct our study, we established the following two hypotheses.

Hypothesis 1: Carnivores are less likely to be eaten as meat than other types of animals.

Hypothesis 2: Larger animals are more likely to be eaten as meat.

We established hypothesis 1 because lions and wolves are not frequently eaten, and other meat is required to feed carnivores. We suggested hypothesis 2 because very small mammals such as mice are not often eaten, while large animals have a considerable amount of meat on their bodies. This study was performed to validate these two hypotheses.

2. Methods

We searched the literature to determine the amount of dog, rabbit, and sheep meat and beef and pork produced in the world [3] [4] [5] [6].

The average weights of dogs, rabbits, cows, sheep and pigs were also obtained [7] [8].

Next, we calculated the correlation coefficient between each animal's meat production and average weight. The reason why we chose to use average weight as an indicator of mammal size is that there are various types of "large" animals: tall, fat, and long. We thought that weight could be used to measure these various types of size.

To evaluate the relationship between animal carnivorousness and their human consumption as a meat source, we ranked carnivores as 2, omnivores as 1, and herbivores as 0 and calculated the correlation coefficient between meat production and these ranks.

3. Results

Table 1 shows each the average weight, carnivorousness rank, and meat production of each animal.

Table 1. Animal weight, carnivorousness, and meat production

	Cow	Pig	Dog	Rabbit	Sheep
Weight (kg)	867.5	140	9.95	1.39625	87.5
Carnivorousness (rank)	0	1	2	0	0
Meat production (ton)	57000000	100000000	320000	8100	32494050

A small but strong correlation was observed between average weight and meat production (correlation coefficient R = 0.394148689).

However, with regard to carnivorousness, there was a negative correlation, but it was not sufficiently strong (R = -0.08793589).

A scatter plot for the relationship between each mammal's average weight and meat production is shown in figure 1. Figure 2 depicts the relationship between the carnivorousness and meat production of each mammal.

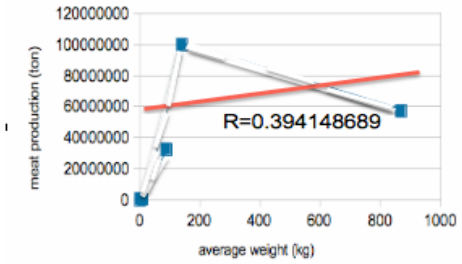

Figure 1. The relationship between average mammal weight (x axis) and meat production (y axis)

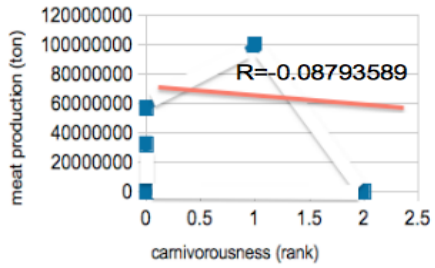

Figure 2. The relationship between carnivorousness (x axis) and meat production (y axis)

4. Discussion

According to figure 1 and the determined correlation coefficient R, bigger mammals have a higher likelihood of being eaten. Meanwhile, there is no relationship between carnivorousness and meat production, as demonstrated by the weak correlation coefficient.

Previous studies have suggested that the nutrition derived from a meat source is one criterion to determine whether it is good to eat. Additionally, we found that the bigger a mammal is, the more likely it is to be eaten as meat.

However, this research has several limitations. Only cow and pig data were included as heavy animal data. Considering this, it is clear that a strong positive correlation would be found between animal weight and meat production because beef and pork are popularly eaten around the world. Additionally, this result cannot explain why rabbit is eaten while elephant is not. We also ignored cultural aspects in this research. Location and religion can strongly affect what meat people eat. Considering these limitations, the meat production of other large animals, such as elephants and bears, should also be evaluated. Investigating the number of countries in which people eat each animal instead of meat production may also be useful for further study.

5. Conclusions

The results obtained suggest that Hypothesis 1 appears to be useless but that Hypothesis 2 may be a new intuitive criterion and is worth studying in detail.

Previous studies have suggested that meat nutrition is a criterion for determining whether a mammal is good to eat. However, it requires us to eat or analyze that meat. By contrast, we provide a new criterion, animal weight, which can be measured without performing a chemical analysis. This is quite useful for hypothesizing what drives the choice to eat or not eat an animal's meat.

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