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Analysis of Rural Ecosystem in Japan Using Stable Isotope Ratio

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1. Introduction

1.1 Rural ecosystem in Japan

Rice is one of the most important cereal crops especially in large part of Asia and a part of Africa. It is split up into two groups. One is upland rice, grown on dry soil. Most rice is grown in flooded rice paddy fields and most of them in Japan are irrigated well. Rice is considered to come to Japan about three thousand years ago from southern China, analyzing Carbon 14 of unearthed rice seed from paddy field remains.

Paddy fields are, so to speak, fabrication of floodplain area. Some areas near river are flooded in rainy season and change into shallow puddles in dry season. Wild rice plant probably has spread in such wet lands. Ancestor reclaimed and cultivated paddy fields and irrigated them after a period of primitive harvest of wild rice.

Paddy fields are also special and sacred place where spirit lives and give us fertility. The spirit is said to live in the deep mountain during winter and come down to communities when spring comes. Farmers welcome the spirit with honor. We can see their soul by devoting a sprig of camellia to the spirit at the inlet of the paddy field when irrigation water inflowed into the paddy field at the beginning of cultivation. Paddy fields have affected traditional human society and life in Japan.

Contribution and influence of paddy fields is not exclusive not only to human beings. The artificial wet lands provide some wild lives good spawning, growing, feeding site. **Photo 1** is scene before puddling, preparation for rice planting. It shows water network between canal and paddy fields. Water level is lower than photo's level in non-irrigation period. So paddy fields are dry in non-irrigation period. A weir which is set at the beginning of irrigation period makes water level high as **Photo 1** and fishes such as loach can swim up into paddy fields in order to spawn. Frogs also benefit from paddy field and canals. Tokyo Daruma Pond Frog (*Rana porosa porosa*) (**Photo 2**) spawns in paddy fields and grows up near canals after metamorphosis. They have been using the new habitats efficiently.

Mosaic land use in rural area is characteristic in Japan. **Photo 3** is typical land use in hilly and mountainous rural area. Of course, there some farming types in Japan, including dairy husbandry, large scale upland crop or rice farming. But hilly and mountainous rural area account for 40 percents of cultivated acreage in Japan. Lower area in hilly and mountainous rural area is used for rice farming, being irrigated from canals. Dry field locates in upper area.

Photo 1. Mt. *Yakeishi* reflected on the surface of the newly irrigated paddy fields, Iwate Prefecture, Tohoku district

Photo 2. *Satoyama* and paddy fields that were poured with irrigation water before puddling

Photo 3. Tokyou Daruma Pond Frog in a paddy field

By National Land Information web mapping system–Ministry of Land, Infrastructure, transport and Tourism

Photo 4. Typical land use in hilly and mountainous rural area

Village forest called *Satoyama* in Japanese had a role of wood fuel and compost production. Underbrush in *Satoyama* had been cut well not to transit into overplanting several decades ago. Fuel revolution and developing pressure altered *Satoyama* into golf course or run-down bush. *Satoyama* was also precious habitat for wild lives similarly as with paddy field and canal are comfortable habitat for fishes and frogs. Mosaic structure help many wild lives adapted to these various environments. Japanese Brown Frog (*Rana Japonica*) move to *Satoyama* after metamorphosis, feeding and overwinter there. Both paddy fields and *Satoyama* are necessary for their life cycle. So, plural mosaic land use aggregate (we can recognize them as each ecosystem)

Photo 5. Drainage canal separated from dual-purpose canal, irrigation and drainage

Thus, rural ecosystem in Japan is influenced by paddy fields that account for half of farming land total. Ecosystem affected by agriculture, especially by paddy fields is called Secondary Nature. A word of secondary may include an inferior nuance. However the term Secondary Nature never has such a nuance. A lot of living lives are seen in a rural area. A number of rare species also survive in rural area with human activities.

Photo 6. Drop work is constructed for moderation of channel slope, opposing swimming up of fishes

The rural ecosystem in Japan has deteriorated during recent years because of land improvement that permitted agricultural production to be efficiently, chemical materials input into paddy fields and development in rural area. Land improvement as a term must be less familiar with general readers. I would introduce farm land consolidation that is main construction project in land improvement to readers. Traditional paddy fields in Japan were so narrow and cultivators had some fields from place to place. Because the same channel had been used for irrigation and drainage, ground water level was so high that farmers had not been able to bring agricultural machines to paddy fields. For, soil bearing capacity was low before independent setting of irrigation and drainage. Dual-purpose canal was separated into irrigation channel and drainage canal by farm land consolidation. As underground drainages are constructed in many consolidation projects, moisture of soil become dry. Japanese Brown Frog that has a habit of coming to paddy fields to spawn from *Satoyama* in early spring, non-irrigated period, lose a chance to spawn in dry paddy fields.

Canal is also renovated from soil to concrete by farm land consolidation. Concrete canal has superiority from the point of agriculture, that is, water management, maintenance. On the other hand, there is inferiority from the aspect of environment. Water velocity becomes large, covering by concrete. Considerable aquatic animals can't swim against velocity after repair. We can see some water drop facilities that adjust gradient and water velocity in complete canal. Even though they are needed to keep canal performance, they prevent fishes from swimming up to upstream. If small animals, for example Japanese Brown Frog that comes from *Satoyama* to spawn, drop into concrete canal, they never escape from concrete canal because they don't have suckers with their fingers like Japanese Tree Frog. Thus, land improvement consisted in such as agricultural canal renovation influenced

habitat of aquatic animals so much, although it gives us a lot of contribution. Technology has both sides, convenience for our ordinary life and harmfulness for ecosystem.

It is only one decade ago that eco-friendly construction seems to be needed in Japanese rural area. Deterioration and damage of ecosystem were the background of revision of Land Improvement Act in 2001. The revision included that land improvement project must attend to harmony with the environment.

However, biology of rural wild lives and how biodiversity could be conserved are not well known. It's even less of a surprise that how we shall do to attend to the environment concretely. Does the facility built in order to conserve wild animals in a canal work well actually? Is the effect of the facility enough to keep the ecosystem? We have to investigate not only technical methods for water facilities but also basic biology of rural living organism and characteristic of ecosystem

2. Biological analysis using stable isotope ratio

Some elements have isotopes that don't emit radiation and are called stable isotopes. Carbon-13 and Nitrogen-15 are two such isotopes often used to analyze a food web. These isotopes inevitably exist in nature, and also are present in the bodies of organisms. Stable isotope ratios for carbon and nitrogen are expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Stable isotope ratio is defined as following formula.

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000(\text{‰})$$

δ : Carbon stable isotope ratio: $\delta^{13}\text{C}$

Nitrogen stable isotope ratio: $\delta^{15}\text{N}$

R_{standard} : in case of C; Carbon stable isotope ratio of Belemnites in PD-stratum, South Carolina

In case of N; Nitrogen stable isotope ratio in Nitrogen gas in the air

R_{sample} : Stable isotope ratio of sample, $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$

Plants are classified into 3 groups by the photosynthesis mechanism, namely C_3 , C_4 and CAM plants. Scarce plant species in the paddy fields of Japan belong to the CAM plant group. A considerable number of plants in the paddy area are C_3 plants, and all trees in forests, *Satoyama*, belong to this group.

Sugarcane, corn, eulalia, crabgrass, and giant foxtail belong to C_4 plants. C_4 plants can often be found in the periphery of a paddy field. They are usually members of Gramineae that are natives of tropical regions. The $\delta^{13}\text{C}$ values for C_4 plants are approximately -13‰, with a range from -15‰ to -10‰. The $\delta^{13}\text{C}$ values of organisms are considerably lower than C_4 plants, approximately -27‰, with a range from -35‰ to -25‰.

The values for algae in an environment that abounds in CO_2 are between C_3 plants and C_4 plants, at approximately -20‰ as shown by previous investigations.

The type of plants present as producers in a food web can be predicted by analysis of $\delta^{13}\text{C}$, because $\delta^{13}\text{C}$ changes little under prey-predator interactions. Therefore, when the $\delta^{13}\text{C}$ of an animal is approximately -30‰, the animal is considered to be in a food web derived from C_3 plants (**Fig. 1**).

Fig. 1. C-N map representing food web

We can see several characteristics in Japanese rural vegetation. One is mosaic botanical distributions. They are paddy fields, fields, grass lands, orchard, forests between mountain and arable area that is called *Satoyama* in Japanese language. It is not uncommon that these distributions are found in even 1km². Only C₃ plants are found in *Satoyama*. On the other hand, significant proportion of plants near paddy fields belong to C₄ plants though rice crop itself is C₃ plants. They often grow in the terrestrial area like land temporarily fallow. Algae are also important producer in rural ecosystem. A lot of aquatic animals feed on algae. Difference of $\delta^{13}\text{C}$ between detritus and algae. Almost detritus in a agricultural drainage canal origins from litter produced in *Satoyama* and paddy fields, where only C₃-type plants grow. The difference still remains at animal level. That is, $\delta^{13}\text{C}$ of animals fed detritus is lower than that of animals fed on algae.

3. Actual investigations

1. Analysis of food web

Using the characteristics of carbon and nitrogen stable isotope ratio described previously, we can analyze food-web. I would like to offer you the analysis example of which sample is collected in rural area. The investigate area was Isawa plain that locates in Iwate prefecture, Tohoku district.

The samples were dredged from two aquatic areas, a drainage canal and a small irrigation pond. The canal had been constructed eco-friendly, of which bottom hadn't lined with concrete. Stones, earth and sand had been deposited to a substantial extent. The pond located on the boundary between plain that and a hill. A lot of leaves have fallen into the pond of which water was brownish-red, probably because of the elution of organic element from leaves.

Fig. 2 shows C-N map (food-web) in the canal. $\delta^{13}\text{C}$ of samples that is the most important value to distinguish food-web was approximately from -25‰ to -20‰. Attached matter that has fed insects, fishes though insects was consisted with detritus and algae according to the microscopic visualization. The value of attached matter confirmed it because the value located between $\delta^{13}\text{C}$ of C_3 plants and that of algae. Of course, we can't afford to ignore the existence of C_4 plants. A basin of the canal was occupied by paddy fields where rice that belongs to C_3 plants grows. The top of food web was Amur Minnow (*Phoxinus phoxinus*). Their stomachs were filled with caddice-worms.

Fig. 2. C-N map of collected matters from a canal

Fig. 3. C-N map of collected matters from a pond

How was the food web in the pond? **Fig. 3** indicated quite lower values than that of the canal. The food web depended on C_3 plant apparently. The top of the pyramid should be small insets, water boatman (*Notonecta triguttata*) that suck fluids of small fishes, tadpoles,

insects, etc. Such a curious food web like this sometimes appears in an aquatic area where no fish live. Bladderwort is insectivorous plant,

2. Analysis of Food source

A loach is one of the most familiar freshwater fish for Japanese. It had been swimming everywhere, so catching it had been common play for children. It has been important food, especially for certain rural area even today. Loaches have been decreasing like other fishes. Revamping of canal much affected their life, because they favor sandy bottom that disappeared after construction.

To increase them is becoming popular in these days, as they are also the feed for white stork (*Ciconia boyciana*, Critically Endangered) and Japanese crested ibis (*Nipponia nippon*, Extinct in the Wild). Loaches that are their feed to release to wild also attract attention. To invest feed of loaches is an essential problem besides environmental improvement.

When irrigation water is poured into paddy fields in spring, loaches begin to swim up into paddy fields, where they spawn. Young fishes swim down to canals in early summer after feeding and growing there. Sampling and analyzing were continued from June to September to grasp the change of $\delta^{13}\text{C}$ of fishes bodies. Sample loaches were caught in a paddy field in Kaminokawa town, Tochigi prefecture. C-N map of the loaches is shown as Fig. 4.

Fig. 4. Seasonal C-N map of loach

Along with the season, stable carbon isotope ratio was decreasing and decreasing. This phenomenon declared that food source of loaches has changed from terrestrial matter to aquatic matter. Along with the season, stable carbon isotope ratio was decreasing and decreasing. Copepod in early phase, bloodworm in late phase may be main feed by consulting the past reference and benthic examination in paddy fields. Another was that trophic level hasn't changed, showing the fish was carnivorous even though loaches are said that they become to be omnivorous with growth.

Secondly, I show the analysis of food resource of shell. *Matsukasagai* shell (*Pronodularia japonensis*), a kind of unionid, is necessary for Japanese bitterling because it has a habit of spawning, inserting their ovipositors into the shells. Extinction of the shell means the same of the fish. The shells are described as they feed plankton, but detail is not well known. So I investigated them using stable isotope ratio.

They suck FPOM (Fine Particulate Organic Matter) with water, filtering and eating real feed. The rest of FPOM is excreted. **Fig. 5** shows the result of investigation including FPOM and their spoor in certain habitat, Nakagawa River basin in Tochigi prefecture, where Tokyo bitterling (*Tanakia tanago*) that was protected species lived. There was a pond in upper stream of the habitat. The habitat is described as Point A in this article. Carbon isotope ratio of *Matsukasagai* shell was low, -32.5‰ . The value of spoor was -28.9‰ , locating right between *Matsukasagai* shell and FPOM. Phenomenon kindred to it was seen in flesh water calm, *Corbicula* sp.. These represented that FPOM contained their feed and unwanted materials were excreted, of which carbon isotope ratio was higher than their feed.

Fig. 5. C-N map in Point A

Matsukasagai shells were caught in Point B near Point A, too. A pond located upstream of point B just like Point A. The result was also similar to Point A, that is The value of spoor (-33.3‰) located right between *Matsukasagai* shell (-39.9‰) and FPOM (-27.0)(**Fig. 6**). The low value of -39.9‰ indicated notable information, considering the origin of the organic matter. C_3 plant and detritus derived from it never indicates such low value. Carbon isotope ratio of plankton may represented the value of -20‰ approximately in a circumstance of which gas exchange is well-balanced, however I suppose that quite little ecosystem in land water wouldn't not be balanced on CO_2 in practice, DOC (Dissolved Organic Carbon) that is resource of photosynthesis of algae including phytoplankton may lower than DOM derived from CO_2 in the air. This might be attributed recycling of DOC in the water, preparation and dissolution, value of $\delta^{13}\text{C}$ might decrease during recycling because of isotope fractionation. **Fig. 7** shows relationship between shell length and $\delta^{13}\text{C}$. $\delta^{13}\text{C}$ value of *Matsukasagai* shells increased with their growth. This suggested that feeding habitat would change as they became larger.

This hypothesis was confirmed to some extent by other analysis shown as Fig. 8. It is about 50km distance from Point A and B to Point C, where *Inversidens japonensis* that is relative species of *Inversidens japonensis* lived. Basin area of Point C was different from that of former two points. Basin area of Omoigawa River in **Fig. 8** is quite distant from A and B even all of them locate in Tochigi Prefecture. Harakawa River locates in Iwate Prefecture, as described before, in a northern direction from Tochigi Prefecture.

Fig. 6. C-N map in Point B

Fig. 7. Relationship between Shell length and $\delta^{13}\text{C}$

Fig. 8. C-N map of bivalve in various habitats

All of these three species are said to be plankton feeders. We can assert with probability that there are characteristics of value of DOC in each aquatic environments.

3. Analysis of animal moving

Using behaviors described previously, we can analyze not only food webs but also ecological characteristic of animals. Japanese brown frog (*Rana japonica*) is typical amphibian in rural area. Their populations are said to be decreasing rapidly after revamping of agricultural drainage canal. Prime cause is presumed that concrete agricultural drainage canal cut network between terrestrial area and aquatic area. According to qualitative knowledge in the past, adults of Japanese brown frog live in *Satoyama* and overwinter there. After coming out from hibernation, they move to paddy fields and lay spawns in paddy fields. *Satoyama* is important habitat for the frogs indeed. But I have also often seen them near paddy fields like land temporarily fallow during my field research. Are their wintering places only *Satoyama*? We need to know how they move to *Satoyama* quantitatively.

As some C₄-plants grow in lands temporarily fallow, $\delta^{13}\text{C}$ of frogs body will higher than value of C₃-plants if they feed in the land. However, catching frogs is too difficult because they move to paddy fields during nights and they have agility. So, I came up with usage of spawns.

Sampling was conducted in hilly and mountainous rural area, where the frogs have preference to live, in Tochigi prefecture (Fig.9). The investigated paddy field A borders *Satoyama* on two sides, fallow lands on two sides. Field B borders *Satoyama* on one side, a fallow land on one side, and other paddy fields on two sides. Over 100 egg masses were skimmed up from each field, using a dipper, and dropped back after being sampled about 20 eggs from every mass. The dedicated small tin cups to analyze were filled with eggs after being defatted by methanol and chloroform, crushed. Stable isotope ratios of the samples were analyzed with a mass spectrometer, Delta plus XP, manufactured by Thermo Finnigan.

Fig. 9. Investigated fields

Fig. 10 shows the total result of analysis. The histogram has two peaks. One peak that of central value is minus 25 per mill indicates their mother frogs fed in the C_3 environment. We can see that another group exists, seeing higher value from -21 to -16 per mill values; their mother's foods included appreciably insects that had fed C_4 plants. The result says that most Japanese brown frogs move to *Satoyama* after metamorphosis or spawn, where they feed and winter.

Then, is there relationship between land use around the paddy field and moving? I estimated that $\delta^{13}C$ value of eggs in field A (**Fig.11**) might be higher than in field B (**Fig.12**) because the former had two borders to fallow lands where C_4 plants grew. But there wasn't considerable difference between A and B. That suggests that frogs in the population move to *Satoyama* at a constant rate.

What does the result mean? Agricultural drainage canals are usually routed through boundary area between paddy fields and *Satoyama*. Most frogs that come to paddy fields to spawn fall easily into a concrete agricultural drainage canal and wouldn't be able to escape from there. Conservation of the frogs must be needed when canal would be revamped not to let the population disappear. The problem is we don't prepare the effective methods actually. Slopes at the canal walls constructed to help escaping from the canal aren't used frequently. Only a few individuals that escaped from the canal used the slopes may not have so important meaning, for most of individuals died in the canal before spawn. Capping on the opening section of canal also is a good method. But it costs a lot and farmers often disagree to cap it because they won't use water to wash their farm tools. Some complain of anxiety that it may negatively affect to rise water temperature though rising water in while flowing through in the canal is nothing much.

Fig. 10. Histogram of $\delta^{13}C$ distribution of spawns (entirety)

Fig. 11. Histogram of $\delta^{13}\text{C}$ distribution of spawns (Paddy field A)

Fig. 12. Histogram of $\delta^{13}\text{C}$ distribution of spawns (paddy field B)

4. Analysis of ecosystem structure

Paddy field at hill-bottom or certain area with it is called *Yatsuda* in Japanese language. *Yatsuda* means the latter here. *Yatsuda* includes paddy fields and *Satoyama*, aquatic area and terrestrial area. And canals almost always run at the border area, where boundary area between Paddy fields and *Satoyama*. I considered that I could comprehend plural small food-webs near canals, which are virtually identical ecosystem for each land use there.

I focused on spiders for the food-web analysis because they located top of a micro tentative food-web. We can divide spiders into two groups, web-builder and hunting, from the point of their life types. Furthermore I separated hunting types into phytophilous hunting spiders and epigaeic hunting spiders because each type fed particular foods, considering their habitat.

I analyzed carbon stable isotope ratio ($\delta^{13}\text{C}$) of spiders (Arachnida) which were caught nearby farm ditches and a levee in a paddy field at hill-bottom (*Yatsuda*) with their life types. $\delta^{13}\text{C}$ of web-builder spiders (**Fig. 13**) was -26.0‰ , which indicated that they have been greatly affected by insects emerged from the ditches that had depended on the organic matter supplied from village forest (*Satoyama*). $\delta^{13}\text{C}$ and its distribution of long-jawed water spiders (*Tetragnatha maxillosa*) was approximately equal to silver vlei spiders (*Leucauge magnifica*). That suggested that both of spiders depended on the same food resources. Although $\delta^{13}\text{C}$ average of phytophilous hunting spiders (**Fig. 14**) was close to the web-building type, the standard deviation was higher than that. $\delta^{13}\text{C}$ of epigaeic hunting spiders was -22.2‰ , which indicated that C_4 plants rose $\delta^{13}\text{C}$ value of the spiders (**Fig.15**). It was suggested that $\delta^{13}\text{C}$ of epigaeic hunting spiders lay in close relation with the rate of C_4 plants to the hole plants on the levee and approximately one third of carbon in their bodies was derived from C_4 plants. Diversity of material flow in creatures by carbon stable isotope ratio in the area of *Yatsuda* ditches was revealed, which is the eco-tone between *Satoyama* and a paddy field (**Fig. 16**). Animals in a narrow area shared foods and are compartmentalization, which will be recognized as diversity of ecosystems. Canals in boundary area between *Satoyama* and paddy fields are often consolidated while land improve project is implemented. Of course, new canals after construction are of concrete. Complex and diversified ecosystem there will be transformed into homogenized one.

Fig. 13. Histogram of web-builder spiders

Fig. 14. Histogram of phytophilous hunting spiders

Fig. 15. Histogram of epigaeic hunting spiders

Fig. 16. Food web and carbon flow around canals in *Yatsuda* area

5. Analysis of population structure
May population of animals be homogeneous? Isn't there revolving of members in the population? Constitution of population is an important issue to sustain it as population with complements will still continue even if members would decrease. Stable isotope ratio gave me a vast amount of help.

Stable isotope ratio of animals has an interesting behavior that is caused by difference of turnover time for their parts. Turnover time of muscles and stomachs is short. On the other hand, that of bone is quite slower than these parts. If there is deference among parts, actually between bone and other parts, there is potential that the frog has fed in strange habitat in past times because carbon isotope ratio of previous food is presumed to remain in bone yet. Material of animal tells where he lived.

Carbon isotope ratio of the three parts of Tokyo Daruma Pond frog (*Rana porosa porosa*) that was caught at *Yatsuda* area was analyzed. The result is shown as **Fig. 17**. We notice that there is variability among the group and within certain individual internally. Specifically, vales of bone of individual number 5 and 19 are lower than that of muscle and stomach. This indicates that the frog has grown at another habitat and joined the population at a later time, remaining particular value of carbon on its bone. Overall values of number 6 and 10 are different from majority individual. This means they joined the group quite recently, thus carbon isotope ratio of the body hasn't changed into background value of catch area (habitat of the population) completely yet. Background value will be affected by weighted average of stable isotope ratio of producer. Number 6 might grow at the circumstances where considerable C₄ plants grow. On the contrary, number 10 might grow at the circumstances where all but C₃ plants grew and it fed insects that depended almost exclusively on C₃ plants.

Fig. 17. Carbon isotope ratio of Tokyo Daruma Pond Frog for each parts

These truths educate us important things. Being consisted with individuals moved from plural habitats is also important obviously from the biology point of view, but how in-migrants joined the group includes internal issue for conservation. The species live near paddy fields and/or canals, into which frogs jump when they are attacked by predators. Some attacked frogs escaped into flowing canal will be washed away to downstream although most frogs will drift to near dropped point. Even the washed frogs try to land, but they won't be able to escape from there, if the canal will be constructed by concrete. Existence of in-migrants is the proof of dropping, being washed and landing of frogs. Canals seem to have a role of pathway of aquatic animals not just a role of agricultural production. Breakpoints of network have harmful effects not only for swimming up of fishes but also accession of frogs from other populations. This is just my own supposition, in-migrants might have a role of gene transfer to certain population.

4. Ecosystem and photosynthesis

Stable isotope ratio has been providing a lot of relevant knowledge about rural ecosystem. To pursue the ecological phenomenon in rural area from the point of biology, it has contributed so much. And findings from the development of ecological engineering are also needed. I'm convinced that stable isotope ratio will help us to conserve rural ecosystem as an evaluation method of the engineering effects.

What thus far discussed depends on the difference between photosynthesis cycle, C_3 plants, C_4 plants and algae. Photosynthesis gives us not only organic matter that is vital for all of animals but also efficient method to investigate and to conserve ecosystem.

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Photosynthesis is one of the most important reactions on Earth, and it is a scientific field that is intrinsically interdisciplinary, with many research groups examining it. This book is aimed at providing applied aspects of photosynthesis. Different research groups have collected their valuable results from the study of this interesting process. In this book, there are two sections: Fundamental and Applied aspects. All sections have been written by experts in their fields. The book chapters present different and new subjects, from photosynthetic inhibitors, to interaction between flowering initiation and photosynthesis.

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