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The Study of the Effect of Invasive Alien Species on Biodiversity (Abstract of the Interim Report)

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Total Budget for FY2001-FY2003   180,314Yen   (**FY2003**   58,590Yen)

1. Introduction

Biological invasion is one of the main factors threatening biodiversity. The ecological impacts caused by invasive alien species (IAS) are very serious because they are increasing and making irreversible change on the natural fauna once they become established in the "new" field.

Though in Japan the number of IAS is considered to be increasing year by year, the study for invasive alien species has been so far behind. It was decided in the COP 5th meeting held in 2000 that the member nations of COP must make up national measures for biological invasion as soon as possible. So, Japan Government legislated new law for IAS Act at June 2004.

However we have little information sources pigeonholed systematically for IAS, yet. And we also have few study case of the process and mechanism of biological invasion. Furthermore, we have already serious invasive alien pest animals and birds, for which an immediate measure for extermination is needed.

2. Research Objective

In the present study, we compile a database of invasive alien species in Japan, which will help us to take proper measures to prevent and control invasive alien species. The schema of the database should conform to that of IUCN-GISP invasive alien species database. We also develop the criteria of invasiveness. For enhancement of counter measures to invasive alien species, this database will be made accessible to the public to get systematic information of invasive alien species.

And in order to clarify the biological mechanism of ecological impacts of invasive alien species on the native, we analyze experimentally the process of competition, predation, genetic introgression, parasite invasion and modification of habitats caused by invasive alien species. For the study, we selected various typical invasive alien species and investigate spatial distribution and ecological niche of the invasive alien species, the status of genetic introgression caused by inter-species hybridization using molecular genetic markers. We also detect parasite organisms from the invasive alien species, and assess the change in quality of habitat where the invasive alien species is distributed.

Furthermore, we study on process of naturalization, population dynamics and control measures of the serious invasive alien animals and birds in order to contribute to the guideline for management of invasive alien species.

3. Methods

For the construction of a database of invasive alien species in Japanese, we selected invasive alien species in Japan that should be listed in the database mainly according to their invasiveness. Data compilation proceeded for these species. A special focus is being placed on providing core background information regarding the ecology of invasive alien species, as well as on ecological and economical damages, legislative control, and control and eradication techniques. Species information includes biology, ecology, distribution (map), management information, references, and images. The database was developed with MS Access 2000(Microsoft Corp., 2000)

For the investigation of biological mechanism of ecological impact of invasive alien species, we selected typical species from birds, reptiles, fishes, insects and weeds, which are introduced into Japan for biological industry. For these species we investigated mechanisms of ecological impacts as below;
- White-eye *Zosterops japonica* (bird): We investigated infestation rate by plasmodium in individuals from various sites of Japan and Hog-Kong using molecular genetic markers.
- Red-billed Leiothrix *Leiothrix lutea* (bird): We investigated ecological niche of the invasive alien species and the native Japanese Bush-Warbler *Cettia diphone* in the forest of Ebino Highland, Miyazaki Prefecture. We also compared the fitness of the native species between in the area where the invasive alien species nests were removed and in the area where those not removed.
- Revee's Pond Turtle *Chinemys Reveessi* and yellow-spotted lance-head snake *Trimeresurus elegans* (reptile): we investigated morphological characters and genetic constitution of hybrids between this invasive and the native species, *Mauremys japonica* and *Trimeresurus flavoviridis*
- Large mouse bass *Micropterus salmoides* (fish): We investigated the population density of the native fishes as a parameter of predation of the invasive alien species in the lakes in Aichi, Gifu, and Hyogo prefectures, where the invasive alien species lives or not.
- Brook trout *Salvelinus fontinalis* (fish): We investigated ecological niche and the status of hybridization between the native fish and the invasive fish in Azusa River, Nagano Prefecture, using molecular genetic markers.
- Mosquito fish *Gambusia affinis* (fish): We investigated the distribution of the invasive alien species and the native species Medaka fish *Oryzias latipes* around Kiso River, Aichi Prefecture. We also experimented competition behavior between the two species using water tanks.
- European bumblebee *Bombus terrestris* and exotic stag beetles *Dorcus* species (insect): We investigated status of hybridization between the native and introduced species using genetic markers at various site of Japan. In order to assess the hybridization risk, we made cross experiments between the introduced and native species. We also checked the parasite infestation in introduced individuals, using molecular genetic markers as well.
- Weeping love-grass *Eragrostis curvula* (weed): We investigated spatial distribution of the invasive alien species and the change of the flora around Kinu River, Ibaraki Prefecture. Based on ecological properties of the alien weeds, we constructed a model for estimating

expands of distribution of the weeds.

For the study of serious invasive alien species, we selected two mammal species and two bird species and investigated as below;

- Melodious laughing thrush *Garrulax canorus* (bird): With questionnaire, the past and recent distribution of the species was investigated. In order to show its habitat preference, point censuses were conducted at forests with and without dense understories. The food menu was clarified and a parasitological survey was conducted to assess the impact on the native bird species. An experimental removal of bushes was carried out in order to control the population densities.

- Red-billed leiothrix *Leiothrix lutea* (bird): We investigated population dynamics at the forest Mt. Tsukuba in Ibaraki using mark-recapture methods and Jolly-Seber model, and did observation of nests. I also collected fecal samples from leiothrix and native bird species and analyze them to describe diets.

- Formosan squirrel *Callosciurus erythraeus* (mammal): The presence data of Formosan squirrels were obtained by questionnaires and field surveys from 2001 to 2002. The habitat preferences of individual squirrels were investigated by radio-tracking in which vegetation surveys have been conducted. The probability of Formosan squirrel occurrence in suburban landscape was analyzed by logistic regression model using environmental variables detected by GIS.

- Raccoon *Procyon lotor* (mammal): We investigated actual distribution in Japan using hunting records and news reports, ecological survey for grasping the influence on native animals by raccoons at the forest in Hokkaido using radio telemetry and auto-camera methods, public consciousness on the invasive raccoon issue in Hokkaido and Kanagawa prefecture, and reproductive characteristics of invasive raccoons in Hokkaido to construct the eradication countermeasure.

4. Results and Discussion

The database contains information on 337 species.   Table 1 shows that the number of species included in the database for each taxonomic group. Species are also classified whether they were internally (within country) or internationally (from abroad) introduced.

Table 1. Number of species listed in the database (total 337species)

Taxonomic Group	Vertebrates					Invertebrates		Plants
	Mammals	Birds	Reptiles	Amphibians	Fish	Insects	Others	Vascular Plants
International introduction	29	39	13	3	44	34	3	98
Internal introduction	6	-	6	10	50	-	-	2
Total	35	39	19	13	94	34	3	100

All taxonomic groups of non-native species and organisms may be included in the database, and be accessible via Internet websites; however, this study was not able to collect information on the whole of over 2,000 non-native species in Japan.

The database contains species information on 337 species regarded as the most invasive ones, and provides data sets by searchable fields including Japanese name, scientific name, English common name, morphological description, native and alien geographical range, invasion pathway, local dispersal methods, management regime, and scientific references

in Japanese. Additionally, picture images and distribution map are provided for some of the species.

Our database structures and query interface are currently formulated by the software design that can be adapted to personal computers. It should, however, provide a web-based query on national and international scale systems for early detection, tracking, and strategic management of IAS in Japan.

Accumulation of case study is needed for typing invasive alien species. In the present study project we study mechanism of ecological impact of typical invasive alien species. We found that predation by invasive alien species cause great decrease of native species through the survey of lakes where the large mouse bass *Nicropterus salnoides* lives in a large number and the native fish density is much lower than in no large mouse bass living lakes.

Inter-specific competition between the native and invasive alien species is considered to cause negative impact on the native species, suggested by the field survey of biological interaction between the invasive and the native fishes in Azusa River (brown trout) as well as Kiso River (Mosquito fish *Gambusia affinis*).

On the other hand we could see habitat segregation between the native and invasive alien species; Red-billed Lethionex *Leiothrix lutea* seemed to use empty habitat in the natural forest. In this case the serious impact cannot be seen directly. However, the field experiments of removal of the invasive alien species nests showed that the existence of alien nests seemed to reduce the fitness of the native bird species because of natural enemy attack. So we must consider as well indirect impact of co-existence of invasive and native species.

We could found many cases of inter species hybridization in the field between invasive and native species using molecular genetic markers; the brook trout, turtles, snakes, and stag beetles. It will be important to conduct a follow-up survey for the genetic introgression as a consequence of the hybridizations, considered as a chronic impact of invasive alien species.

On the other hand we found that crosses between the European bumblebee *Bombus terrestris* and the Japanese native species can make fertilization but cannot make hybrid maybe because of reproductive isolation. This means the possibility of reproductive disruption through inter species copulation, which is a new concept of ecological impact of invasive alien species.

Furthermore, phylogenetical analysis and cross experiments of the stag beetles indicated that there seemed to be little isolation between genetically and geographically far isolated strains. The Indonesian and Japanese *Dorcus titanus* strains considered to be isolated for 5 million years could copulate and produce a lot of F1 hybrids (fig.1) and as well F2 hybrids. On the contrary, crosses between genetically and geographically closer strains showed reproductive isolation to a high degree (Fig.2). This suggested that genetic and geographic distance between the species is not always in correlation with the degree of reproductive isolation, which would be a complicated aspect of genetic problem caused by invasive alien species.

We found many parasitic organisms from introduced individuals. Plasmodium species were detected from the White eye from Hong-Kong, and parasitic mites detected from the European bumblebees from the Netherlands and the stag beetles from the south-east Asia. Furthermore, field survey showed that the mites of the European bumblebee had started to spread among the native species populations in Hokkaido. And molecular genetic

analysis suggested a close co-evolution relationship between the stag beetle and mite. Disruptions of such a relationship caused by artificial transportation may result in unexpected ecological impacts. These parasite investigations strongly suggested importance of quarantine systems.

The field survey around Kinu River showed that the weeping love grass had changed the rock-based river-bed to be sand-based, which reduced the native plant populations. This indicates the difficulty of recovery of native flora once invasive alien species invade the habitat. The spatial distribution dynamics model of the weed based on survey of its ecological properties and distribution pattern indicated its strong potential to expand its distribution invasively.

These empirical results will be important base for ecological risk assessment of countless invasive alien species, collaborated with the database described above.
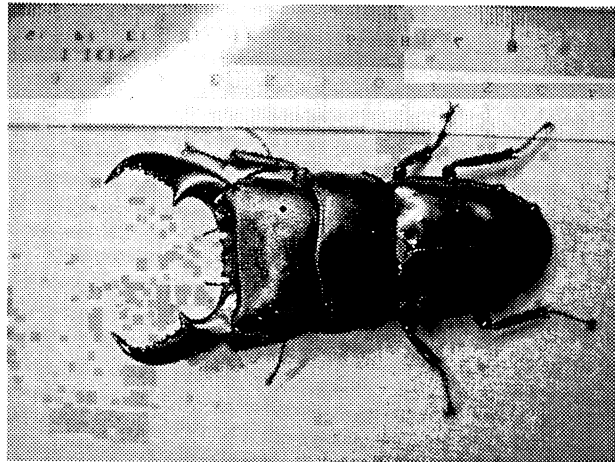

Fig.1. An adult male of F1 hybrid between the Indonesian female and Japanese male Hirata stag beetle, *Dorcus titanus titanus* and *D. titanus castanicolor*. Its body size (85 mm) is much larger than its father (50 mm).
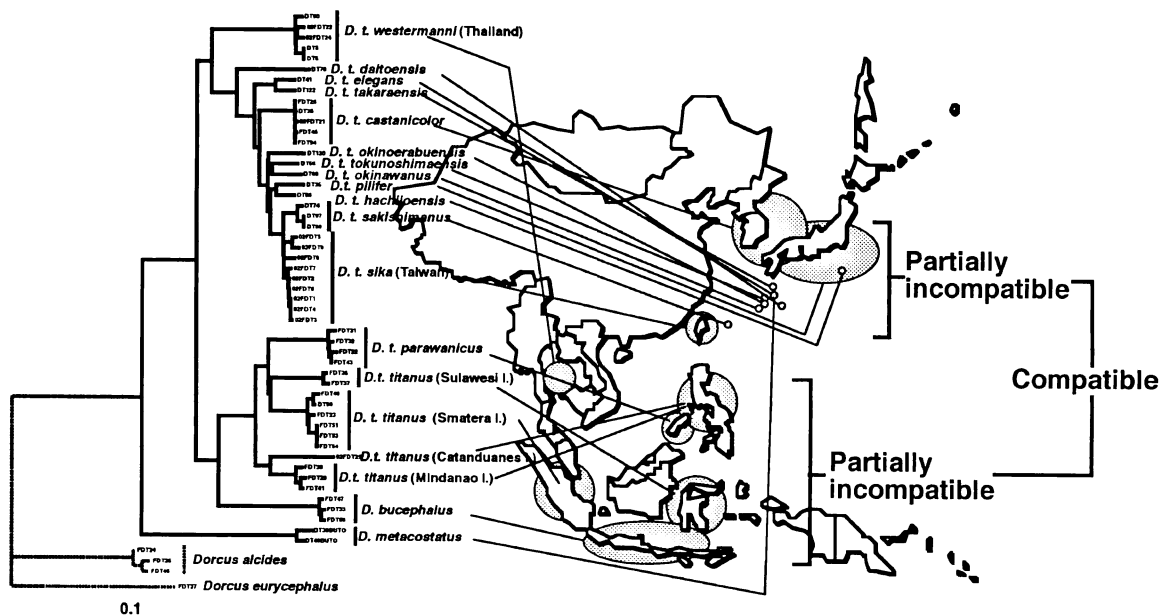

Fig. 2. The phylogenetic tree of the Hirata stag beetle *Dorcus titanus* species group, and the genetic compatibility between them.

For some serious invasive alien species we need to develop effective measures to control. In the present study we investigate ecological aspects of the four important invasive alien species. The distribution of the feral Melodious laughing thrush *Garrulax canorus* in Japan was clarified. There were four populations of the species in the Western Kanto area, the Northern Kyushu area, Nagano prefecture and Fukushima prefecture. It mainly occurred below 1000 meters elevation and preferred a snow less area. No serious impacts on the native bird species were detected for now. The distribution is, however, still expanding, the species might impact on them in future. The species preferred dense understories at broad-leaved deciduous forests, and the population densities can be controlled with removal of thickets.

Demographic parameters of an introduced population of Red-billed leiothrix *Leiothrix lutea* on Mt. Tsukuba during 1991-2001 were estimated by mark-recapture methods. Mean population size of adult birds was 205.6±34.3 and showed no significant trend of increase through the study period. Mean annual survival rates were relatively low, 0.395±0.054 for adults and 0.206±0.043 for juveniles. Breeding success of introduced Red-billed leiothrix on Mt. Tsukuba was studied during 2000-2002. Active nests were found from April to October and clutch size was 3.4±0.9 (mean±SD). Fledging success in the three years were 22.6 14.9, 45.3 %, respectively. Most failures were caused by nest predation. Although nest failure was frequent, annual breeding performance of each pair might be improved by repeated nesting trials during the long breeding season. Fecal samples from Red-billed leiothrix contained plant seeds more frequently than in those from Bush Warbler. Bush Warbler depended more on spiders than leiothrix. Competition for food between the two species seems most likely to arise in early spring when both depended on Hymenoptera and Coleoptera.

The distribution area of the Formosan squirrel *Callosciurus erythraeus* expanded to 304 km² in 2002. Their expansion process during 52 years fits to the model expected by the exponential population growth. We should control 5-10% of present population to prevent further increase. They prefer to use the vegetation with various tree species and dense canopy, so forest treatments such as thinning and weeding may be effective in suburban isolated woodlots. In order to prevent intrusion of the Formosan squirrel to the habitat of endemic species, the large forests located along the Sagami River and the Sagami Bay seem to be most urgent.

The distribution of the invasive raccoon *Procyon lotor* almost spreads throughout the whole country and invasive raccoons have a wide variety of habitat, from forest to urban area. The competition with native raccoon dog and the predation on indigenous species such as Japanese crayfish and Ezo salamander became clear. The invasive raccoon is opportunistic and omnivorous in feeds, taking agricultural products in farming area and native animals and plants in forests, so the eradication countermeasure should be changed according to the actual damage condition of the each area. As for the method of capturing, multiple capture method in the rearing period will be effective. Almost people of the damaged area agree to the extermination of the invasive raccoon, but we should make greater efforts to inform the serious damage on biodiversity by invasive alien species.

These results seem to make it possible to estimate the change of distribution and dynamics of the invasive animals and to offer the ideas to make agreement among local community for the management.