

Meso-scale Structure of Population and Community
Associated with Behavioral Traits in Insects

January 2017

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Associated with Behavioral Traits in Insects

A Dissertation Submitted to
the Graduate School of Life and Environmental Sciences,
the University of Tsukuba
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Science
(Doctoral Program in Integrative Environmental Science)

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Abstract

In the field of ecology concerning with scales, indices and models for estimating higher order structures from lower order structures are precedent, and to collect practical examples for making them more accurate is an urgent matter. In this study, meso-scale was assumed to be the spatial extent that each species can potentially reciprocate within a lifetime, that is, at least two subpopulations within a meta-population are included in it. Population and community structure was observed on various temporal and spatial scales by tracking survey of individuals. It is aimed to extract elements which are stored in higher order scales. The study animals are the insects which mainly inhabit and move in planar space such as water surface (sea-skater (Gerridae: *Halobates*)) and ground surface (carabid beetles (Carabidae)).

Sea skaters, genus *Halobates* are the only marine insects living in mangrove beds and the tide pools of coral reefs, both of which provide dramatically variable habitats daily by virtue of tide-driven changes in surface level. Females of *H. japonicus* were collected from three distinct aggregations in a single bay. Amplified fragment length polymorphism markers were used to detect the spatial persistency of each aggregation at a meso-scale. The results suggested that aggregations along the coastline had some persistency to the line itself and though they had little tendency to cross to the opposite shore, even when the distance was short.

Vegetation structure provides the habitat template for the assembly of carabid beetle species in micro scale, and meso scale characteristic of terrain and spatial arrangement of agricultural elements including artificial constructions has formed a mosaic of those vegetation patches. The study was carried out in a rural landscape at elevations around 780 m of the cool temperate zone of Japan in short summers, July and August, of 2007-2010. Vegetation structure and some terrain

or soil characters of four study sites were measured. At each stand, 144 (=12 rows×12 columns) pitfall traps were set and the carabid beetle assemblages were caught. Non metric multidimensional scaling and regression models showed that carabid beetle community structure at each stand mainly defined by stand age and its cedar forest structure of micro scale, though, the species composition was influenced by spatial and temporal meso-scale factors.

Elevational pattern of species richness and diversity was determined in carabid beetle assemblages inhabiting artificial cedar forests of a rural landscape. Adults were collected by pitfall traps installed along an altitudinal gradient (815, 835, 870, 915, and 955m). A total of 13 species belonging to four subfamilies were identified from 363 collected ground beetles. Non metric multidimensional scaling and regression models highlighted that the diversity of carabid beetle might be determined by the elevation band, the angle of slope and vegetational structure of the forest floor. Direction of short-term individual movement was different for each species, and it is also suggested to create elevational pattern.

Historically accumulated factors can be reserved in the DNA information of individuals at temporal macro scale or mega scale, and researchers can give suggestion about the dynamics of population at higher temporal scales using DNA from the spatial meso scale sampling when the meso scale is properly set on each species. Capture and recapture method can only have a temporal micro scale information, so spatial micro scale survey together with spatial meso scale survey can be helpful to determine the size of population and the dynamics of community. Thus, spatial meso scale study based on the behavioral traits of each species could be valid in creating a picture of transitional zone of the hierarchical structure ecosystem.

Key Words: amplified fragment length polymorphism, artificial cedar forests, carabid beetle community, diversity, meso-scale, rural landscape, sea-skater population, species richness

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General Introduction

Factors determining animal mass structure such as population and community include historically accumulated ones and short term ones with random fluctuation. The native effect and successional changes are included within the former phenomena, while ecological equivalence, patch dynamics and disturbance are included in the latter (Delcourt et al., 1983).

Historically accumulated factors can be observed in the information as DNA of individuals because they have repeatedly worked as a directed selection force to each individual. They also can be reserved in the higher layer in hierarchical structure of animal mass.

In principle sampling scale has to be selected according to the scale of which the reaction of biota occurs. On a two-dimensional time-space graph, each reaction of biota have the region as shown in Fig. I which was marked out by Delcourt et al. (1983) based on the plant communities. Considering on the studies of mass structure of insects, observation methods have been biased toward either micro scale or macro scale. The studies on population are included in the micro scale such as competition, production and gap dynamics (Atkinson & Shorrocks, 1981; Beyene et al., 2007; Gibbs & Dyck, 2009). Their spatial units are at least from 10 m to 100 m and their temporal units are 1 d or longer. On the other hand, studies on community structure are included in the macro scale whose spatial units are at least from 100 m to 1000 m, and temporal unit is more than one month (Scott & Anderson, 2003; Yu et al., 2006; Wiezik et al, 2007). Some studies suggested that the animal mass structure along a certain environmental gradient sometimes showed the opposite trend to the known one because they had differences in spatial scale of sampling (Menin et al., 2007). Thus, for the last decade, researchers have paid attention to the transitional zone between micro scale and macro scale, meso scale, especially in spatial

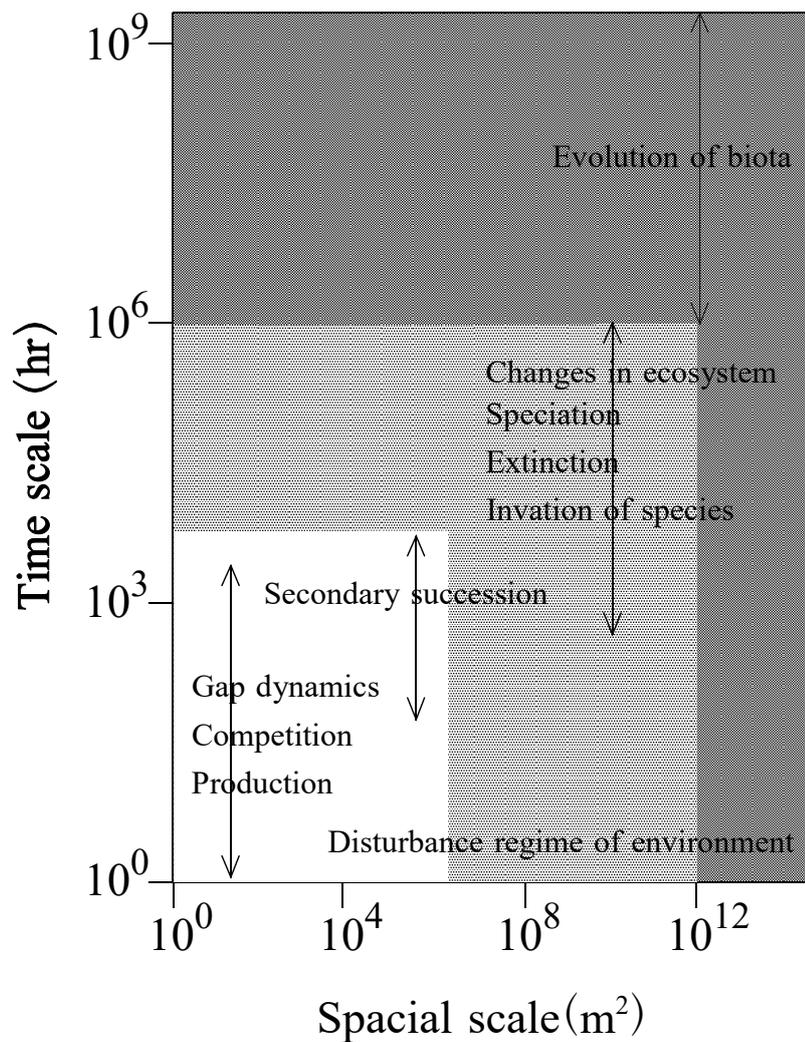


Fig. I Reaction of biota to environmental changes mapped on two axes of time and space (Delcourt et al., 1983; Turner et al., 2001). White colored area, gray area and dark gray area represent micro scale, macro scale and mega scale, respectively.

scale.

The problem of scale is also found in the way how the existing indices have been used in each field researcher. Community ecologist and conservational ecologist have intensively applied Shannon-Wiener's diversity index (Shannon, 1948) for local communities until recently in order to describe relatively higher mass structure. The index was simply accepted by most field researchers because it was helpful for roughly figuring out the structures in the field. However, as numerous studies piled up, field surveys whose species richness had deviation from the calculated value of the diversity index had often been found. Then some researchers especially from the landscape ecology suggested that species diversity must be described using the present-absent data of species.

In the field of ecology concerning with scales, indices and models for estimating structures of higher layer from the structure of lower layer are precedent (Turner et al., 2001). Collecting practical examples to make them more accurate ones is an urgent matter. Especially, it is necessary to get the picture whether each phenomenon which is observed in the lower layer of structure is observed in the higher layer. In the process of revealing this, observation on an intermediate scale is indispensable. A number of studies have already looked at the meso-scale (0.5 to 10^4 km²) patterns of mass structure in plants (Clark et al., 1998; Niemelä, 1999; Wiens, 1997), and the key words "meso-scale" have been found a lot in 1990's studies, however, there had been a few example of meso-scale studies on animals until 21st century.

Animal mass structure in lower layer had been affected from the structure in higher layer, and vice versa. Recent meso scale studies suggest that the most species-rich areas in a region are those with a high degree of environmental complexity, both within and between habitats

(William & Marilyn, 1999). Predator-prey interactions in the presence of meso scale barriers were also discussed (Walters et al., 1999), and models for predicting the higher mass structure from the behavior of the elementary units had already been made in marine life.

Population and community structure of terrestrial animals are relatively defined by the behavioral traits more than the marine life, which means that marine lives tend to be influenced by the meteorological disturbances more than terrestrial lives when they are in the same order of body size. In the same way, species in the small order of body size tend to be influenced by the disturbances more than the larger sized species. Furthermore, animals cannot change their body size after growing up, and the size of their mouth also cannot change, which means that the amount of food intake per unit time is also limited for adults. This naturally causes trade-off between the energy for body maintenance, production and dispersion (Begon et al., 2006). The migration or dispersion will influence both habitat selection of each individuals and the rank of each species in a community, which might provide direct changes in population and community structures. Thus, the meso scale for land-dwelling animals might be smaller than that for terrestrial plants (Delcourt et al., 1983).

In this study, meso-scale was assumed to be the spatial extent that each species can potentially reciprocate within a lifetime. That is, at least two subpopulations within a meta-population are included in the meso-scale of each species. Specimens in this study are the insects which mainly inhabit planar space, sea-skaters (Gerridae: Halobates) and carabid beetles (Carabidae). On the observation of sea-skater, when a certain time is cut out in a natural population, the group size and the boundary are clear, but tracking observation is difficult, so we tracked the movement of the individual retrospectively using genetic information. Whilst the

group size and the boundary of carabid beetles are unclear when a certain time is cut off, but follow-up observation is possible using the method of capture and recapture (Southwood & Henderson, 2000). Also because of easy accessibility to anywhere in the study area in any time, field survey can be set in various spatial and temporal scales, which will be helpful for getting information of transitional zone.

CHAPTER I

Topological persistency found in aggregations of coastal sea skater, *Halobates japonicus* Esaki, 1924 (Hemiptera: Gerridae), based on genetic relationships among three aggregations in Ishigaki Island, Japan

ABSTRACT

Sea skaters, genus *Halobates*, are the only marine insects living in mangrove beds and the tide pools of coral reefs, both of which provide dramatically variable habitats daily by virtue of tide-driven changes in surface level. Females of *H. japonicus* were collected from three distinct aggregations in a single bay (24°27'50"N, 124°8'40"E) on 1-5 November 2006. Two aggregations were formed in respective tide pools of the coral reef and one was formed in the mangrove beds. Amplified fragment length polymorphism markers were used to detect the persistency of each aggregation at a meso-scale. The results suggested that *H. japonicus* repeatedly immigrated from outside the bay to establish a meta-population of several deme groups. Aggregations along the coastline had some persistency to the line itself and though they had little tendency to cross to the opposite shore, even when the distance was short.

INTRODUCTION

Animals form aggregations in the air, on the sea, on vegetation, and elsewhere, for purposes such as reproduction, feeding, and/or avoiding predation (Bertram, 1978). Most aggregations are maintained by the equilibrium of recruits and dropouts, for which the persistency of individuals to a certain aggregation is of intrinsic importance (Pulliam, 1988).

Some marine invertebrates are exposed to inevitable dispersion because their bodies are small enough to be caught in the high viscosity of sea water (Andutta et al., 2012; Haase et al., 2012), thus, their persistency becomes an even more important factor for maintaining aggregations.

Representatives of the genus *Halobates*, wingless sea skaters, belong to five species of the open ocean and 41 species inhabiting coastline. Most of the coastal 30 species occur in the tropical parts of the Indo-West Pacific Region, roughly between latitudes 30°N and 30°S (Andersen & Cheng, 2004). Aggregations of coastal species have been well studied in endemic *Halobates* spp. in the Galapagos Archipelago (Foster & Treherne, 1980) and in the Seychelles (Cheng 1991). It was shown that to avoid predation, these aggregations disintegrated right after a predatory attack (Treherne & Foster, 1981) and that most of them formed again at the same locations repeatedly (Treherne & Foster, 1982). The aggregations also function as arenas for a mate selection and re-aggregate in less than one minute very close to the shore after being dispersed. In contrast, aggregations of juvenile insects stay away from the shore and do not re-aggregate for more than one and a half minute (Foster & Treherne, 1982). Birch et al. (1979) also described the persistency of aggregations of *Halobates robustus* Barber, 1925 in relation to the daily changes in the coastal line, where aggregations oriented themselves to topographical features irrespective of the water surface film movement by wind and/or the tide. Though

significant barriers to gene flow have been suggested at a macro scale, due to the low frequency of dispersion limited by physical barriers (Leo et al., 2012), there is no quantitative information on genetic flow at a meso-scale.

In Japan, Ikawa et al. (2012) reported that *Halobates japonicus* inhabits coral areas along the islands in the southern parts of Japan (Fig. 1-1). Although the daily tidal changes made it difficult to observe a particular aggregation continuously during the day, I nevertheless demonstrated that *H. japonicus* showed behavioural persistency to a certain aggregation (Ikawa et al., 2006). In the present study, this persistency based on genetic relationships between populations were attempted to be measured using amplified fragment length polymorphism (AFLP) primers.

Information from genetic markers has been frequently used to infer gene flow and migration tendencies of individuals among populations (Slatkin, 1985; Cockerham & Weir, 1993; Falush et al., 2007). AFLP markers have been commonly used to differentiate breed varieties (Bensch & Åkesson, 2005) and have been applied to insects to reveal interactions among deme groups (Timmermans et al., 2005; Parkes et al., 2009; Ben-Shlomo & Inbar, 2012). *H. japonicus* adults were sampled from all aggregations in a single bay where they could still disperse passively by currents (Fig. 1-2). Among these aggregations, only one survey site was substantially far from the other sites and, thus, provided favourable conditions to detect the persistency of individuals based on information about their genetic and topological relationships.

MATERIALS AND METHODS

Three aggregations of *H. japonicus* were collected from Kabira Bay, Ishigaki Island, on 1-5

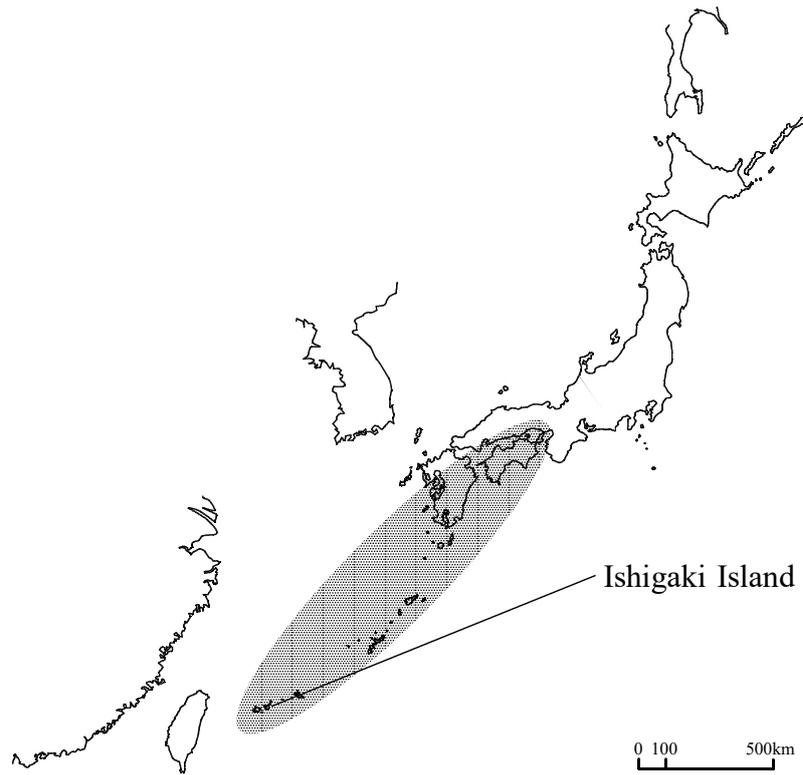


Fig. 1-1 Geological outline map showing the Japanese Archipelago. The shaded area represents the distribution of *Halobates japonicus* including Ishigaki Island at the southernmost part of Japan and also of the distribution area (Andersen and Cheng 2004).

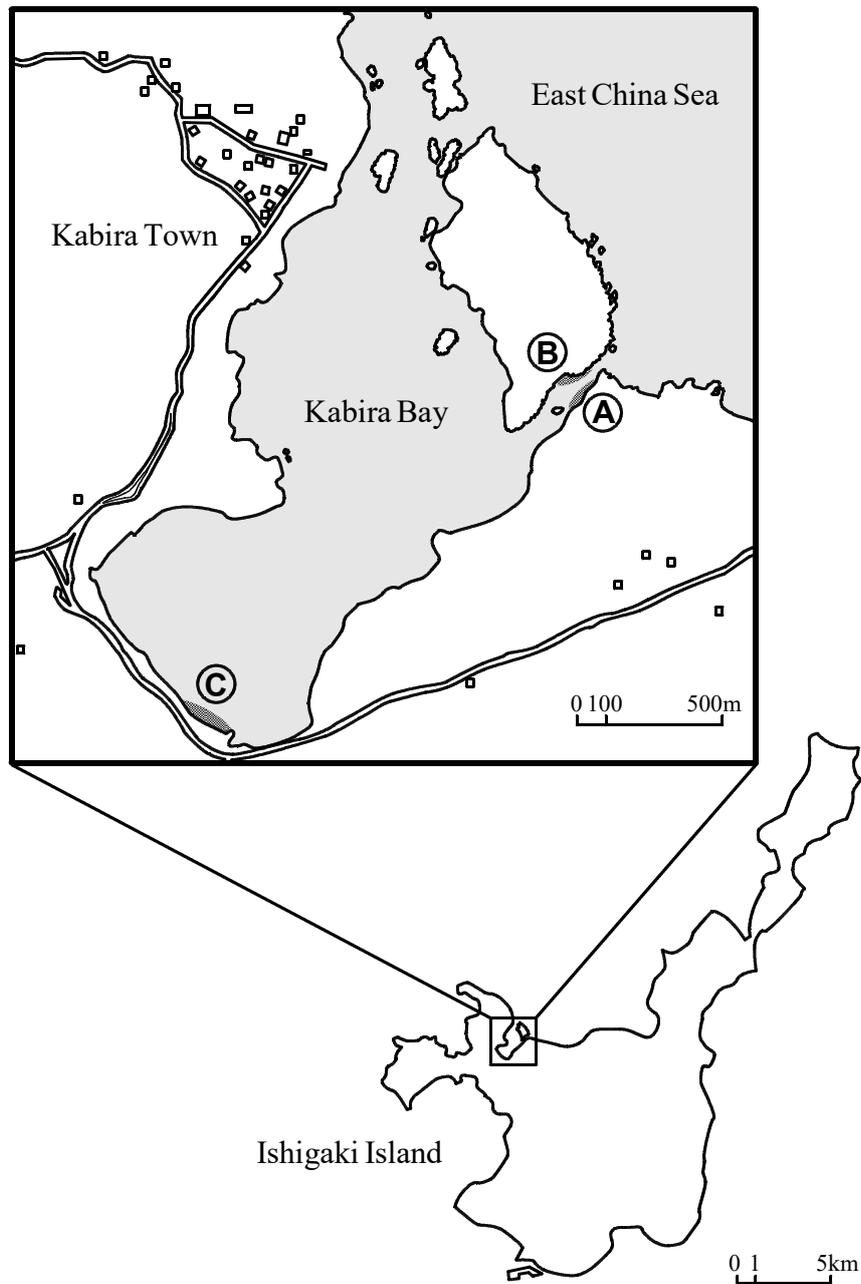


Fig. 1-2 Map showing Kabira Bay, west of Ishigaki Island, Japan. The three dotted areas represent shallow coral areas that included the survey pools for sites A and B out of numerous tide pools. The substrate in the survey pool of site C was mud, not coral, due to its location along a mangrove forest. Squares on the land indicate houses.

November 2006 (Fig. 1-2). Two of them were located in the tide pools on both sides of a canal (site A, 15×40 m; site B, 5×20 m), which was wedged between the sheer rocky cliffs (ca. 5 m height) with overhanging tree crowns of dwarf oak, *Quercus phillyraeoides*. The habitat of site C (6×10 m) was located in a tide pool along a mangrove, *Avicennia marina* in the closed-off section of the bay. All habitats were connected to each other twice a day at full tide. An additional survey from 29 October to 3 November 2007 was conducted, and aggregations of all sites were observed indicating the current speed as an environmental factor. Female sea skaters, 25 (site A), 14 (site B), and nine (site C) individuals, were collected using nets during one sampling period. Males were collected only from site A during the sampling period. They were used in order to select the primer set for *H. japonicus* but were excluded from the latter analyses.

DNA was isolated from one hind leg of each adult using a DNeasy Extraction Kit (Qiagen, Crawley, UK). PCR reactions were conducted according to Vos et al. (1995) using the protocol for the AFLP Core Reagent Kit (Invitrogen, Carlsbad, CA, USA). The sizes of amplified fragments, ranging from 60 to 350 bp, were determined through fragment analysis with a CEQ 8000 genetic analysis system (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's protocol. The presence of fragments was scored 1 for band presence and 0 for band absence.

A hierarchical analysis of molecular variance (AMOVA) was performed to assign components of variation to the sample level (ARLEQUIN version 3.5.1.2). Genetic distances between pair-wise combinations of the populations were expressed as F_{ST} values. Nei's (1972) gene diversity statistics in the different aggregations was estimated using POPGENE version 1.32 (Nei, 1987; Francis & Yang, 2000). Banding patterns were also analyzed using

STRUCTURE version 2.3.4 (Pritchard et al., 2000). STRUCTURE uses a maximum likelihood approach to determine both the most likely number of distinct genetic groups in the sample (K) and which individuals are most likely to belong to each group.

RESULTS AND DISCUSSION

At site A, some aggregations consisted of adults (82.6%) and late instar larvae (17.4%); they repeatedly vanished and regrouped within a few hours. The aggregation of site B was composed of adults only, while site C was composed of adults (87.5%) and larvae (12.5%). Current speed on the coral area (site A) was 0.006-0.000 m/sec (SE, n=9) and that on the shoreline along the mangrove forests (site C) was 0.003-0.000 m/sec (SE, n=3) in the same season of 2007.

Two primer combinations were selected from 14 combinations that were analogous to ready-made markers, as reported by Cervera et al. (2000). Fourteen combinations were used for test samples from three sea skater aggregations (Table 1-1). The primer combination of *EcoRI* + *AGG/MseI* + CTC produced 125 markers and *EcoRI* + *AGG/MseI* + CAT produced 198 markers (323 in total), ranging from 60 to 360 bp in length. These markers represented individual differences. No markers were fixed for sex.

Females contained 289 polymorphic markers (89.5% of the markers from females; 75.1% of the markers including both females and males). Within the aggregation at site A, 67.5% of AFLP markers were polymorphic; the aggregations at sites B and C had 53.9% and 45.5% polymorphic markers, respectively. The Nei's gene diversity values (Nei, 1978) for site A individuals (0.193 ± 0.012 , SE) were slightly lower than those of site B (0.209 ± 0.013 , SE) and site C

Table 1-1 Fourteen selective primers used for test samples that were analogous to ready-made markers for *Bemisia tabaci* (Hemiptera), as reported by Cervera et al. (2000).

Primer Pair + Selective Ext.	
<i>Eco</i> RI + AAG	/ <i>Mse</i> I + CAC
	/ <i>Mse</i> I + CAG
	/ <i>Mse</i> I + CAA
	/ <i>Mse</i> I + CTT
<i>Eco</i> RI + AGG	/ <i>Mse</i> I + CAC
	/ <i>Mse</i> I + CAG
	/ <i>Mse</i> I + CAA
	/ <i>Mse</i> I + CTT
<i>Eco</i> RI + AGC	/ <i>Mse</i> I + CAC
	/ <i>Mse</i> I + CAG
	/ <i>Mse</i> I + CAA
	/ <i>Mse</i> I + CTT
	/ <i>Mse</i> I + CAT
	/ <i>Mse</i> I + CTC
<hr/>	
<i>Eco</i> RI + AAG	/ <i>Mse</i> I + CTG (Cervera et al., 2000)
<i>Eco</i> RI + ACT	/ <i>Mse</i> I + CTG (Cervera et al., 2000)

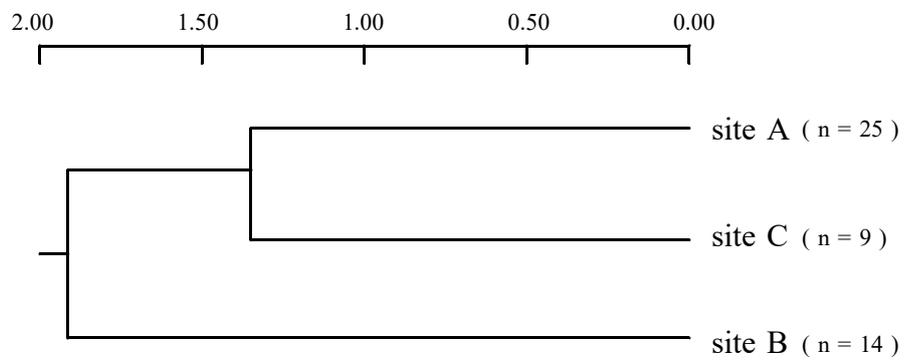


Fig. 1-3 Dendrogram showing genetic relationships amongst three aggregations using the unweighted pair-group method with arithmetic mean UPGMA based on Nei's (1972) genetic distance. The letters on the right-hand side of the dendrogram each represent a site with sample size (n).

(0.205 ± 0.013 , SE) individuals. Genetic diversity was higher when the samples of all sites were combined (0.222 ± 0.011 , SE). AMOVA showed that approximately 86.0% of the variation in the data set was derived from genotypic variation within aggregations (Table 1-2). The genetic divergence value for the three aggregations combined was 0.14, indicating that there was low differentiation between aggregations.

The genetic relationships among the three aggregations were calculated using Nei's (1978) genetic distances (*D*) and genetic identity (*I*) (Table 1-3). The largest genetic distance was found between the aggregations of sites B and C, and the lowest genetic distance was found between those of sites A and C (Fig. 1-3). Unlike the case with genetic distances, the topographical distance between sites A and B was less than 50 m, and the mangrove bed containing site C was at least 1.5 km from the coral area (Fig. 1-2). Although the relationship between the genetic and topographical distances was uncertain due to the small number of sites, two processes could be predicted to explain this mismatch. Either there is some kind of strong linkage between sites A and C, or there are some physical barriers (e.g., currents, tides, and wind) or some ecological barriers, which might be stronger between sites A and B than between sites A and C.

Aggregations of coastal sea skaters show some persistency in their specific near-shore location irrespective of the tidal movements (Birch et al., 1979). The biological roles of aggregation have been studied in relation to predator avoidance (Foster & Treherne, 1981; Treherne & Foster, 1980; 1981; 1982), feeding (Foster & Treherne, 1980), and reproduction (Foster & Treherne, 1982); and in all cases, the aggregation tended more or less to maintain itself even after physical or ecological disturbances. The populations of sites A and B had

Table 1-2 Analysis of molecular variance based on 404 AFLP loci that quantified the genetic variation among individuals within aggregations and among aggregations.

Source of variation	d.f.	Sum of squares	Variance components	% of total variance	F_{ST}	P value
Among aggregations	2	1392.0	3.23	14.0	0.14	< 0.001
Within aggregations	899	17835.0	19.84	86.0		
Total	901	19227.0	23.07			

Table 1-3 Genetic distances among the three aggregations, based on Nei's (1978) unbiased measurements.

Aggregation	Site A	Site B	Site C
Site A	–	0.964	0.972
Site B	0.036	–	0.945
Site C	0.029	0.057	–

Above diagonal: Nei's genetic identity (I)

Below diagonal: Nei's genetic distance (D)

actually been observed during each of the 5 d. of investigation in 2006 and also during a preliminary investigation in 2005 (Ikawa et al., 2008).

Some reports refer to the migration rate and direction of the locomotion of the sea skater aggregations. Foster & Treherne (1980), during a one-hour observation period, found that approximately 20% of the individuals left their group and did not return. In the open ocean, populations of *Halobates sericeus* were estimated to have low migration rates, up to 0.2 individuals per generation (Leo et al., 2012). Birch et al. (1979) described one aggregation occurring 34 m from the nearest fixed object on the shoreline, but most of them stayed along the edge of the mangrove forest even at low tide. In the present study, tide pools of sites A and B formed directly under the overhanging trees on each shore and were completely separated by sand when the tide was out (Fig. 1-2). Thus, the populations of the sea skaters at sites A and B maintained their topological positions relative to the shoreline at any tide level. Because the direct interchange between sites A and B requires dispersion through open water, it may be that the movement along the shoreline between sites A and C occurs more frequently.

Non-independent pairs of bands were scored as single traits by the analysis using STRUCTURE. We performed five independent runs for $K=1-6$ using 1×10^6 MCMC iterations after a burn-in of 1×10^5 , using the correlated allele frequencies model and assuming an admixture. In the analysis, replicate runs for each value of K (from one to five) were highly concordant in their log-likelihood values (range $\pm 0.0-5.9$, SE), but there was large variance when $K=6$ (± 91.4 , SE). The highest log-likelihood value was associated with $K=5$, indicating that the three aggregations contained five genetically subdivided population groups (Table 1-4). Each aggregation also contained individuals from the five subdivided populations (Fig. 1-4),

Table 1-4 Inferring the average value of K , the number of populations, for the *Halobates japonicus* data

K	$\log P (X K)$	$P (K X)$
1	-3809	0.0000
2	-3429	0.0000
3	-3211	0.0000
4	-3147	0.0000
5	-3081	0.9999
6	-3107	0.0000

The value in the last column was calculated from Bayes' Rule assuming a uniform prior for K ($K \in \{1, \dots, 6\}$).

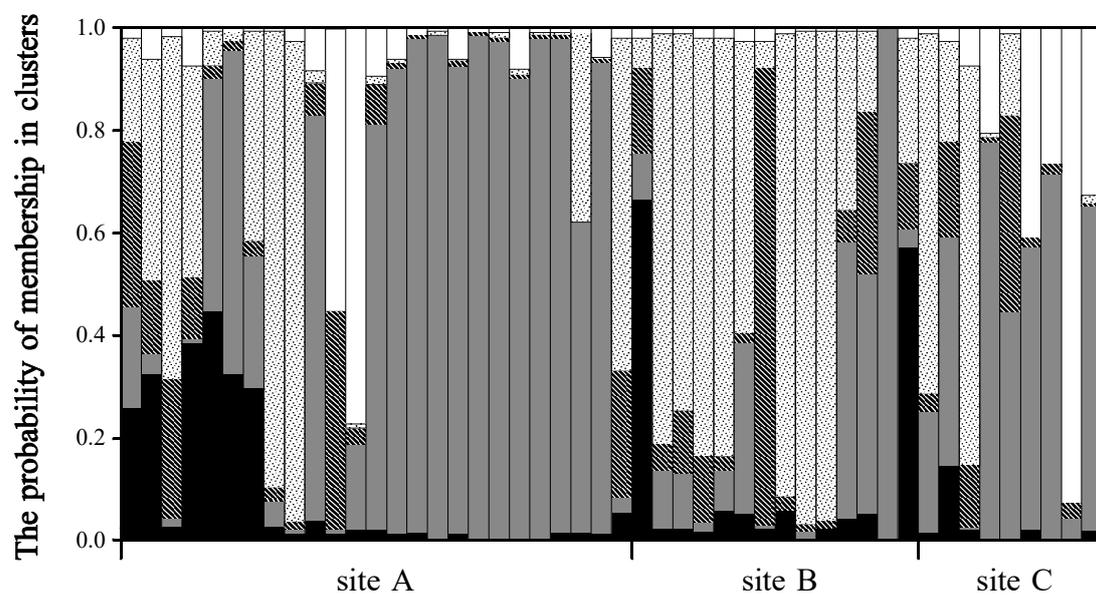


Fig. 1-4 Recommended clustering results ($K = 5$ clusters) using the STRUCTURE program (Pritchard et al. 2000). Each individual is represented by a vertical line that is partitioned into white, dotted, shaded, grey, and black segments. The length of each patterned segment indicates the probability of membership in different clusters.

suggesting that at least one generation had passed from the last subdivided population group that either occurred outside the Bay or immigrated from outside of it.

The present study is the first report of relationships among aggregations of coastal sea skaters distributed in a single bay. We used the AFLP method for genetic analysis and found a high level of polymorphism. Although AFLP is now considered less reliable because of low reproducibility (Pompanon et al., 2005), as is also the case with RAPDs (Perez et al., 1998), the method is still the most convenient in that populations can be genotyped easily at a low price. The 323 polymorphic loci that were produced from two combinations of AFLP markers were sufficient to examine genetic structures among a few groups within a species (Campbell et al., 2003). The results of the STRUCTURE analysis supported the hypothesis that each of the three aggregations in the bay was derived from a number of genetic populations. Repeated immigrations from outside the bay might have occurred via coastal currents or the Kuroshio Current, producing a genetic admixture. However, the study site is in the southern limit of the *H. japonicus* range of distribution, and it is unlikely that the northward-flowing Kuroshio Current brought individuals or groups of *H. japonicus* into the bay. Furthermore, divergence within aggregations was higher than that among aggregations (Table 1-2). This suggested that several deme groups might have been contained within a single aggregation and that all deme groups were common to the three aggregations (Fig. 1-4). Thus, relatively ancient groups might have been detected in this study by the STRUCTURE population analysis, corresponding to the well-mixed meta-population structure in the whole bay (Table 1-2).

The coastal species of *H. japonicus* is diverged from mangrove species (Andersen & Cheng, 2004) and observed in the mangrove forests as well as along coral or rocky coasts. Although the

water current along coral or rocky coasts is faster than that in the mangrove forests, the sea skaters stay at a certain location by the help of topographical features. In the present study, it is noteworthy that the persistency of *H. japonicus* to the shoreline was at the level of the changing genetic structure of the meta-population within a single bay. This characteristic found in each of the aggregations (Birch et al., 1979) might be one of the barriers in life history that separates coastal species from oceanic species. Further experiments on genetic samples outside the bay and on another coastal species are needed for studies of the relationship between behavior and genetic structure.