

Studies on population dynamics of the larch sawfly,  
*Pristiphora erichsonii* (Hartig) during an outbreak in  
the University of Tokyo Hokkaido Forest, central  
Hokkaido

(北海道中央部に位置する東京大学北海道演習林  
の大発生期におけるカラマツハラアカハバチの個  
体群動態に関する研究)

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Studies on population dynamics of the larch sawfly, *Pristiphora  
erichsonii* (Hartig) during an outbreak in the University of Tokyo  
Hokkaido Forest, central Hokkaido

by

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## ABSTRACT

The larch sawfly, *Pristiphora erichsonii* (Hartig), is known to be cosmopolitan species. Outbreaks of this insect were reported in Eurasia and North American continents, and Japan. Its population outbreaks are known to persist over long periods. In Hokkaido, larch trees are non-native species that were artificially introduced from the mainland of Japan and Krill Island, Sakhalin and the Eurasia Continent. Outbreak records of *P. erichsonii* in Hokkaido updated in 1977. In this dissertation, population dynamics of *P. erichsonii* and mortality factors were investigated to clarify reasons why *P. erichsonii* outbreaks continue so long compared to other forest defoliators. The study was conducted at eight stands of larch plantation, seven *Larix kaempferi* stands and one stand of *L. gmelinii* var. *japonica* and a hybrid (*L. gmelinii* var. *japonica* x *L. kaempferi*) in the University of Tokyo Hokkaido Forests (22,717 ha), located in central Hokkaido Island, Japan from 2009 to 2012. Both field and laboratory studies were carried out for investigating population density, natural enemies, and host tree response against herbivory.

Two types of canopy photos, normal color and infrared red, were taken in June and October to determine defoliation intensity. Five litter traps, 1 m x 1 m each, were deployed on forest floor to collect fallen insect frass drops as an indicator of defoliation. Severe insect defoliation was found at two sites in 2009 and at all stands of *L. kaempferi* in 2010 and 2011. In 2012, severe defoliation was found only at one stand among the eight. One stand, where conspicuous defoliation was observed in all the four years, seemed like an epicenter. Although, population outbreaks of *P. erichsonii* were believed to continue for many years in each area at a landscape level, this study first revealed that, at a stand level, the high density with conspicuous defoliation do not continue throughout the outbreak period.

Because the current generation's cocoons are indistinguishable from previous generations' cocoons and small mammal predation had started before the sampling, it was difficult to estimate the number of newly spun cocoons and predation by small mammals with high precision. Therefore, in this study, a hierarchical Bayesian model was developed to estimate these values by one-time sampling of soil every year. Ten 0.04-m<sup>2</sup> soil samples were annually collected from each site in mid-October. The abundance of unopened cocoons (*I*), cocoons emptied by small mammal predation (*M*), and empty cocoons caused by something other than small mammal predation (*H*) was determined. This study estimated the abundance of newly spun cocoons, the predation rate by small mammals before and after the cocoon

sampling, and the annual remaining rate of empty cocoons in the soil using the hierarchical Bayesian model. The model had acceptable fit: a posterior predictive check yielded a Bayesian  $P$ -values of 0.54, 0.48 and 0.07 for  $I$ ,  $M$ , and  $H$ , respectively. The  $I$  value were found peaked in 2010 or 2011 depending on a site and decreased in 2012 with one exception, whereas the  $M$  and  $H$  tended to increase year after year.

Abundance of small mammals was determined by snap traps. The abundance declined in winter until 2009. However, after 2009 when the recent *P. erichsonii* outbreak started, small mammal abundance did not decrease in winter, most likely because the high density of cocoons acted as a supplemental diet during the winter and improved winter survivorship of the small mammals. As a result, a significant numerical response to the cocoons was found in *Apodemus argenteus*, *A. speciosus* and *Myodes rufocanus bedfordiae*. The predation rates by small mammals were also significantly influenced by abundance of these small mammals and year but not by cocoon density itself. On the other hand, overall, the percentage of cocoon killed by parasitoids and entomophagous fungi was low. Neither any significant spatial density dependence was found for parasitic wasps, parasitic flies, nor entomophagous fungi.

Responses of the Japanese larch (*Larix kaempferi*) to defoliation by *P. erichsonii* were examined from a perspective of a carbon/nutrient balance hypothesis (CNBH). This study was conducted in seven stands with Japanese larch. The chemical and physical properties of the foliage were determined from 2010 to 2012. A decrease in foliar nitrogen and increases in phenolics, tannins, and the CN ratio were found in the years following severe defoliation and were significantly influenced by the 2009 defoliation intensity. The influence of defoliation in 2010 and 2011 was weaker probably due to strong defoliation in almost all sample trees. These results indicated that the past defoliation history additively affected the foliage properties even in the two years following insect defoliation. In addition to the 2009 defoliation effects, site effects were found on phenolics, sugars, and the CN ratio. The CN ratio was high at both sites where severe defoliation was found in 2009. Phenolics and sugars did not increase linearly with the CN ratio, indicating some limitations other than available carbon resource in their synthesis. These results suggest that the induced changes in *L. kaempferi* properties are partially up-regulated under nitrogen limitation but that secondary compound synthesis was, most likely, influenced by external site-dependent factors other than nitrogen limitation.

Previous insect defoliation may alter foliage quality, which in turn affects the performance of insect in either the current or subsequent generations. After severe defoliation, not only decreased in foliar nitrogen and related increase of defensive secondary compounds but also food shortage were found, which were likely causes of body size reduction of *P. erichsonii*. Forewing length of *P. erichsonii* females was also measured as a parameter of body size in this study. Adult body size decreased greatly from generation 2009 to generation 2012. In generations 2010 and 2011, the reduction of body size was influenced by both food deterioration and food shortage. On the contrary, generation 2012, the body size reduced greatly without food shortage.

In conclusion, only in a small epicenter, *P. erichsonii* population densities exhibited sustained type of eruption. From the epicenter to peripherals, an outbreak type gradually changed from sustained type to pulse type. A hierarchical model provided reasonable approximations to observed values for number of newly spun cocoons and the number of cocoons preyed on by small mammals. The percentage of *P. erichsonii* emergence decreased year by year. Small-mammal predation increased year by year and attributed to the reduction, however, it could not depress *P. erichsonii* outbreaks at high density. Some other natural enemies sometimes acted as density dependence way, but not strong enough to regulate population outbreaks of this species. Changes in food quality induced by previous defoliation by *P. erichsonii* may have a substantial effect on the population cycle of it. Also, the past defoliation history additively affected the foliage properties. Reduction of the adult body size, caused by food deterioration, was likely to reduce fecundity and initial density of the next generation. However, the interaction proceeded slowly. These are likely causes of sustained type of outbreaks at a landscape level.

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## LIST OF ABBREVIATIONS

UTHF	the University of Tokyo Hokkaido Forest, the University of Tokyo
LP	A plantation at lower altitude (420 m ASL)
HP	A plantation at higher altitude (550 m ASL)
LN	A natural forest at lower altitude (410 m ASL)
HN	A natural forest at higher altitude (680 m ASL)
NIR	Near-infrared
<i>I</i>	The abundance of unopened cocoons
<i>M</i>	The abundance of cocoons preyed on by small mammals
<i>H</i>	Empty cocoons caused by something other than predation by small mammals
<i>N</i>	Newly spun cocoons of <i>Pristiphora erichsonii</i>
$\varphi$	The annual remaining rate of cocoons
$\theta$	The predation rate by small mammals before October sampling
$\kappa$	The predation rate by small mammals after October sampling
$\rho$	The predation rate by small mammals throughout the cocoon period
PDA	Potato dextrose agar
LSF	The larch sawfly, <i>Pristiphora erichsonii</i>
PW	Parasitic wasp
PF	Parasitic fly
EF	Entomopathogenic fungi
UN	Unknown
SMP	Small mammal predation
Aa	<i>Apodemus argenteus</i>
As	<i>Apodemus speciosus</i>
Mrb	<i>Myodes rufocanus bedfordiae</i>
Sorex	<i>Sorex</i> spp.

C	The concentrations of carbon
N	The concentrations of nitrogen
DF09, DF10, DF11	Defoliation intensity in the year 2009, 2010, 2011
LM	Linear model
LMM	Linear mixed model
CNBH	Carbon/nutrient balance hypothesis



## CHAPTER 1: GENERAL INTRODUCTION

Understanding the population dynamics of defoliating forest insects has long been a fascinating issue due to its economic or ecological significance (Baltensweiler et al. 1977; Elkinton and Liebhold 1990; Graham 1956a; Ives 1976; Wallner 1987). The larch sawfly, *Pristiphora erichsonii* (Hartig), is a major defoliator of deciduous conifers belonging to the genus *Larix* throughout the Northern Hemisphere (Drooz 1960; Muldrew 1956; Turnock 1960). North America has experienced *P. erichsonii* outbreaks simultaneously over vast areas (Graham 1956a). The magnitude of this destruction is hard to comprehend, but enormously valuable (Ives 1976). *P. erichsonii* has specific feature that the population outbreaks continue for long compared to other forest defoliators (Kamata 2002; Lynch 2012). The impact of repeated severe defoliation over consecutive years on tree growth was thin crowns, branch dieback and growth loss in larger trees whereas small tree may die (Ives and Prentice 1958; Richmond et al. 1995). Dendrochronological studies, dating of annual ring, also supported *P. erichsonii* was the most important regulator of stand dynamics of *Larix* (Busque and Arseneault 2005; Girardin et al. 2002).

### Theory of population dynamics

Studies of the population dynamics are based on many theories and assumptions, which proposed by various scientists over the years (Begon and Mortimer 1981). The first principle of population dynamics is widely regarded as the exponential (logarithmic) or Malthus law, as modeled by the Malthusian growth model (Ginzburg and Colyvan 2004). Many ecological field studies have attempted to describe the factors responsible for population fluctuations. Although much has been accomplished since Malthus essay on the principle of population in 1798, the task on explanation why population's size changes with time remain a complex challenge (Moreau 2004).

Population regulation underlies most other ecological problems of interest. It is also integral their interest to evolutionary biologists since the major selective forces shaping life histories and behavior, for example, affect the population dynamics (Murdoch 1994). Over the years, two main processes (endogenous and exogenous) have attempted to explain how and when they achieved the regulation. Populations are reacted by their own and in the vital rates on life histories and feedback from the

environment. Such those of feedback, may involve time lags, are termed endogenous (density dependent factors). In contrary, exogenous refer to environmentally related density-independent factors that can be affect population densities (Rookwood 2006).

The issue of different process theories of population regulation has been debated by Nicholson and Andrewartha & Birch (Solomon 1957). Nicholson is usually credited the view of population regulation by density-dependent mechanisms (Begon and Mortimer 1981). His view supported the simple idea that Elton wrote “It is becoming increasingly understood by population ecologists that the control of populations is brought about by density dependent factors”. By contrast, Andrewartha and Birch stated that these factors are not important for population regulation (Turchin 1995; Turchin 2003) and together with Den Boer idea that density independence alone could explain the achieved population regulation (Mueller and Amitabh 2000). The consensus of opinion now accepted that population regulation cannot occur without density dependence (Hassell 1998; Mueller and Amitabh 2000; Turchin 1995).

Population densities of organisms are never truly stable, but uprising begins with some low density and then fall to approximately their original size (Wallner 1987). They may exhibit a remarkable spectrum of dynamical behavior such as stable equilibrium points, stable cyclic oscillations between two population points and stable cycles (May 1975). However, the population were maintained within certain definable upper and lower limits over a period of time by the actions of abiotic and/or biotic factors (DeBach 1964).

Berryman (1987) and Isaev and Khleopos (1977) have independently made an attempt to classify population outbreaks of insects. Sharov (1997) summarized and simplified these theories by summing up into four types if each of high and low equilibrium points is stable or unstable. 1) Cyclical eruption: Both the equilibriums are unstable. An outbreak duration is short (2-3 generations) with 8-11 year cycle. The cycle is often assumed to be driven by mortality due to abiotic factors, 2) Pulse eruption: The low equilibrium is stable. A duration of each outbreak is short and with a pulse like cycle at each locality. 3) Permanent eruption: High equilibrium point is stable. The permanent eruption was sometimes found in invasive species such as pine wilt disease carried by the pinewood nematode in non-native area. 4) Sustained eruption: The population is sustained both at low and high stable equilibriums.

## Researches on population dynamics of forest defoliating insects

Long-term study is needed for better understanding of population fluctuation (Baltensweiler 1971). So far, there are still relatively a few long-term dataset in natural insect population. Forest defoliating insects show a greater tendency toward cyclic behavior. For example, Kendall et al. (1998) revealed that only 17% of the 69 surveyed insect species significantly exhibited periodicity. Among those, five species out of 11 (=45%) forest defoliating insects exhibited significant periodicity (Liebhold and Kamata 2000).

The larch bud moth, *Zeiraphera diniana* is known for its periodic devastation of the subalpine larch-cembra pine forests and for the longest actual population dataset among forest defoliating insects (Baltensweiler and Fischlin 1988). Each local population exhibits a typical pattern of 8-11-year cycle. The basic regulatory mechanism in this cycle was the defoliation-induced change in food quality. Especially, the raw-fiber concentration of needle increased during the outbreak and had a negative feedback on larval survival and female fecundity for the two or more subsequent generations. Synchrony at a valley (landscape) scale and travelling waves at an Alpine arc (regional) scale, have been observed in the cycles at different scales. The spatial synchrony is considered to be caused by spatial correlations in the environment (the Moran effect) (Moran 1953), and/or dispersal (Fischlin 1983; Kendall et al. 2000; Liebhold and Kamata 2000). At the Alpine arc scale the presence of travelling waves could generally be confirmed (Baltensweiler and Rubli 1999; Bjørnstad et al. 2002). Bjørnstad et al. (2002) have demonstrated travelling waves from west to east along the Alpine arc. On the other hand, Johnson, Bjørnstad & Liebhold (2004) argue that the larch bud moth dynamics can be well explained by the epicenter hypothesis, in which population outbreaks spread out from two epicenters in the center of the Alps and the south-west of the Alps, respectively.

The gypsy moth, *Lymantria dispar* L., is probably a species which was most intensively studied among forest defoliating insects because it was intentionally introduced to the US in 1868, established the population, started to increase its density and to expand its range with devastating broadleaved forests in the NE US. In its original distribution in Asia and Europe, *L. dispar* shows a cyclical eruption (Kamata 2002). On the contrary, in N America, the timing of outbreaks has been irregular and difficult to predict (Mcmanus and Csóka 2007). Campbell and Sloan (1978) concluded that the gypsy moth populations in N America exhibited numerically bimodal (sustained eruption) that density dependent processes maintain

stability at both innocuous and for long periods. However, (Elkinton and Liebhold 1990; Liebhold 1992) concluded that the gypsy moth populations in N America exhibited a pulse eruption: at low population density, the population can persist for many consecutive years until some perturbation (usually mass immigration) elevating the populations to high densities. In the release phase of cycle, the populations expand rapidly into outbreak phase, which may persist 1-2 generations.

Population outbreaks of *P. erichsonii* tend to last for long compared to other forest defoliators (sustained eruption) (Kamata 2002; Lynch 2012) with a range of 2-18 years according to dendrochronological analysis (Lynch 2012). Outbreaks are strongly synchronous both in local and regional scales (Nishimura and Laroque 2010). Populations have increased in especially favorable centers and have spread out during a pre-outbreak period. Ultimately, all favorable types in a locality became involved (Graham 1956a). It was believed that population outbreaks of *P. erichsonii* last for many years at landscape and regional scale. However, there were no studies that quantified defoliation or larval density at a stand level.

### **Life history of *Pristiphora erichsonii***

*P. erichsonii* (Hymenoptera: Tenthredinidae) is a univoltine species. Timing of adult emergence differs among locations and years depending mostly on temperature. In UTHF, adults emerge from late June through early August (Panisara Pinkantayong, personal observation). Less than two percent of the emerging adults are male (Turnock 1960). The adult male is 5-9 mm long, with yellowish antennae and cylindrical shaped abdomen with an orange abdominal band. The female, which is 6-9 mm long, has black antennae and body. The abdomen has a broad orange band and tapers sharply towards the rear (Fig. 1-1A).

The reproduction is accomplished without mating. Two or three days after emergence, the adult females start to lay eggs into new long shoots of larch by a saw-like ovipositor. Eggs are translucent, small and hidden by host tissue (Fig. 1-1B) soon after oviposition but rapidly swell to a cylindrically oval shape approximately 1.5 mm long (Ives 1976; Lejeune 1947). The females in field population had a reproductive capacity ranged from 39.5 to 66.5 eggs (Heron 1955) and the mean number of deposited eggs on *L. kaempferi* was 38.1 eggs per female (Higashiura 1988). Newly hatched larvae of *P. erichsonii* have a brown head and cream-colored body. Four molts occur during the larval stage. The young larvae feed

gregariously (Fig. 1-1C) but become relatively solitary in later instars (Turnock 1960). They feed on the needles of the spur shoots but never on those of the long shoots. Larvae typically consume mature needles lasts about 20 days under field condition (Heron 1951). Their feeding period is from July through early September in the UTHF (Panisara Pinkantayong, personal observation). Fully grown larvae have shiny black head and body becomes gray green. The full-grown larva is about 16 mm long, then falls onto the ground and spins papery brownish cocoon in the soil for overwintering as prepupa (Fig. 1-1D) (Drooz 1960; Lejeune 1947). The cocoon is capsule shaped and varies in size from 8 to 11 by 4.7 mm. The cocoon period is approximately 10 months, but certain individuals undergo a prolonged diapause of more than 1 year in the larval stage in cocoons (Drooz 1960). The percentage of individuals undergoing prolonged diapauses was <1% in the UTHF. The molt from the fifth instar to pupa begins approximately 1 week before the adult emergence, so *P. erichsonii* spends as the fifth instar for most of its cocoon period (Panisara Pinkantayong, personal observation). Life history of *P. erichsonii* in UTHF is shown in Fig.1-2.

#### **Previous studies on mortality factors of *Pristiphora erichsonii***

The economic importance of *P. erichsonii* defoliation and a long period of outbreaks have stimulated researcher to examine the fluctuation of populations and determine mortality factors that influence their populations. Both biotic and abiotic factors are capable of changing the population of *P. erichsonii* (Turnock 1960).

#### **Biotic mortality factors**

##### **Pathogen**

Since the middle of 20<sup>th</sup> century, several studies have been reported insect pathogens played a role in natural control of *P. erichsonii*. It was attacked by a number of disease-producing organisms. In Canada and the United States, five genera of fungi (*Isaria*, *Beauveria*, *Spicaria*, *Hirsutella*, and *Empusa*) parasitic on *P. erichsonii* were reported. During 1948-1952, approximately 16,000 field-collected larvae were examined. The larvae 0.2-1.5% were infected by *Beauveria* spp., whereas mortality of 11,000 cocoons ranged from 2.8% to 23.5% (MacLeod and Heimpel 1955). Similarly, in Minnesota, Drooz (1960) recovered 3 genera of fungi, which are capable of killing the *P. erichsonii* cocoons including *B. bassiana*

(Bals.) Vuill, *B. globulifera* (Speg.) Pic., *B. bassiana* (yellow strain), *I. farinose* (Dicks.) Fr., and *Spicaria* sp. In Cumberland, England, *I. farinosa* could be an important factor in the control of *P. erichsonii*, because 25% of cocoons were parasitized under forest conditions (Hewitt 1912, cited in MacLeod and Heimpel 1955).

Bacteria that caused mortality among feeding larval period were also reported (MacLeod and Heimpel 1955). The observation of 5,200 larvae in Ontario from 1949 to 1952 found that mortality due to bacterial infection ranged from 0.6% to 2.0% under field condition. Most dead larvae were infected by *Bacillus cereus* Fr. and Fr. A few strains of bacteria belonging to the families Micrococcaceae, Bacteriaceae, and Bacillaceae were isolated. Other bacteria including a species of Enterobacteriaceae, *Serratia marcescens* Bizio, was also pathogenic for *P. erichsonii*. Two bacteria in the genus *Bacillus* were cultured from cocoons in the Minnesota. One resembling *B. cereus* strain and the other could not be identified, but it was a gram-variable spore former in pure cultures with subterminal spores oval to cylindrical (Drooz 1960).

An infection by the microsporidia, *Thelohania pristiphorae*, apparently hereditary and a reduction in the number of eggs laid per female, was observed in population of *P. erichsonii* in north western Quebec (Smirnoff 1966). The average number of eggs per healthy female varied from 52 to 59, and from 22 to 40 for females containing spores of the microsporidia (Smirnoff and Chu 1968).

No viral infection in *P. erichsonii* has yet been found (Bird 1955). It seems that the importance of pathogens in regulating field population of *P. erichsonii* was low. Furthermore, the absence of pathogen in papers representing long-term population studies of the *P. erichsonii* in outbreak areas, such as the Lake States and southeastern Manitoba, is further confirmation of unimportance pathogen in the population dynamics of this insect (Graham 1956b; Ives 1981).

#### Parasitoids

Many reports have studied roles of parasitoids in population regulation during outbreaks of *P. erichsonii*. In 1910-1913, *Mesoleius tenthredinis* Morley was introduced from England to reduce *P. erichsonii* population during outbreaks in Canada (Muldrew 1955). Initially, it seemed likely that this parasitoid might have controlled the *P. erichsonii* because it became established and no serious damage was caused

by *P. erichsonii* until 1938. In the early 1940s, when the population density of *P. erichsonii* started to increase, it became apparent that the effectiveness of *M. tenthredinis* had decreased greatly (Muldrew 1956). It was found that the parasitoid eggs deposited in host larvae were encapsulated by phagocytic blood cells, which prevented hatching (Muldrew 1953).

Due to the decline in the effectiveness of *M. tenthredinis*, during 1939-1942 two other parasitoids were released in the attempt to control *P. erichsonii*. These were the tachinid, *Bessa harveyi* (Tnsd.) and the pteromalid, *Trineptis klugii* (Ratz.) (Lejeune and Hildahl 1954). *B. harveyi* appeared to be an important factor in decreasing populations. However, the percentage of parasitizing larvae was not more than 60%. *B. harveyi* is poorly synchronized with host life cycle, a great percentage of adult emergence of *B. harveyi* occurs after most *P. erichsonii* larvae have entered the ground and spun cocoons. It appears likely that *B. harveyi* has not been effective during the *P. erichsonii* outbreaks. On the other hand, parasitism by *T. klugii* has been found low or absent (Harman 1971; Muldrew 1956).

Consequently, the other parasitoid species were sought. The ichneumonid, *Olesicampe benefactor* Hinz, has been released against *P. erichsonii* since 1961 (Turnock and Muldrew 1964). At initial release 61% of hosts were parasitized. Percentage parasitism was recorded as high as 87% during 1962-1963 (Muldrew 1967). Because *O. benefactor* appeared to be effectively controlling *P. erichsonii*, it has been redistributed into several locations in North America. In 1975, *O. benefactor* was transferred to Pennsylvania (Drooz et al. 1985). Unfortunately, a hyperparasitoid, *Mesochorus dimidiatus* Holmgren, increased following the introduction of *O. benefactor* (Thompson et al. 1977). *O. benefactor* is host specific but the hyperparasitoid, *M. dimidiatus*, is not. Therefore, the population of hyperparasitoid can remain during low parasite population levels and reduce effects of *O. benefactor* as a parasitoid when *P. erichsonii* populations increase (Unger 1992). Furthermore, small numbers of other insect parasitoids were found from field-collected cocoons. These were the eulophid, *Dahlbominus fuscipennis* (Zentt.), and the ichneumonid, *Eclytus ornatus* Holmgren, *Aptesis indistinct* (Provancher), and *Euceros* sp. However, the total parasitism of those never exceeded a fraction of 1% (Reeks 1954).

The long term studies on the principal parasitoids of *P. erichsonii* were conducted by several researchers. During 1956-1972, life table study plots were established in Manitoba to study *P. erichsonii* population dynamics and impact of parasitoids on population trend. Two introduced parasitoids, *O.*

*benefactor* and *M. tenthredinis*, were important additions to the mortality factors killing *P. erichsonii*. *O. benefactor* displayed a form of density dependence in cocoon stage. However, density dependence was also appeared to exist between *O. benefactor* and its hyperparasitoid, *M. tenthredinis* (Ives 1976). In US, Krause and Raffa (1996) presented the relationship between parasitoids and *P. erichsonii* cocoon abundance. Over 65% of the 18,315 cocoons sampled were killed. The parasitism rate declined significantly with cocoon abundance.

### Predators

Predation by small invertebrate predators acts in several developmental stages of *P. erichsonii*. Three pentatomid species, *Apateticus bracteatus* Fitch., *Podisus* sp., and *Euschistus* sp., have been found feeding on *P. erichsonii* larvae but they appeared the limited value as controlling factors (Muldrew 1955).

Anthocorid bugs, spiders, mites and lacewing larvae were recorded feeding on eggs and the first instar, whereas wasps have been observed decapitating and carrying off the late instar (Muldrew 1956). During the cocoon stage, some mortality can be attributed to various Coleopterous predators. Three species of elaterid larvae, *Ctenicera triundulatus* (Rand.), *C. propola* (Lec.) and *C. resplendens aerarius* (Rand.), preyed on *P. erichsonii* cocoon (Turnock 1960) but the predation rate was low (Muldrew 1955). Carabid and staphylinid adult were also suspected as predators but no positive information is available. Insect predation on the cocoon stage did not appear to be an important mortality factor (Turnock 1960).

Various vertebrates as predators of *P. erichsonii* have been mentioned by many researchers. However, the most intensively studied group of vertebrate predator was the small mammals preying on the cocoon stage. Buckner (1956) described the important small mammal species in eastern Manitoba. These were two species of shrews and two species of voles, including the cinereous shrew, *Sorex cinereus*, the saddle-backed shrew, *S. arcticus*, the red-backed vole, *Clethrionomys gapperi*, and the meadow vole, *Microtus pennsylvanicus*. Mortality due to the predation was exerted between September and October. Up to 90% of cocoons were destroyed by shrew, whereas vole destroyed cocoon <20%. Although over 70% *P. erichsonii* cocoons were destroyed by small mammals (Buckner 1956), it nevertheless appeared no consistent relationship to cocoon densities of *P. erichsonii* and considered to be density-independent manner (Ives 1976). Similarly, Krause and Raffa (1996) presented the relationship between cocoons and



rodents in United States and found increased predation rate under heavily infested trees though they did not vary with *P. erichsonii* densities.

Some previous studies have suggested that bird predation may be an important factor affecting the *P. erichsonii* adults and feeding larvae. In Manitoba, collections of birds for gizzard analyses were made. Forty-three of 54 species of studied birds fed on *P. erichsonii*. The utilization of *P. erichsonii* varied between and within bird's taxonomic group. However, *P. erichsonii* adults were preyed on in proportionately greater numbers than larvae (Buckner and Turnock 1965). Both numerical and functional responses of bird predators appeared to *P. erichsonii* populations (Buckner 1967). Positive functional responses were recorded for 22 species, negative for four species, and no response for two species. The remaining bird species were collected only at one of the two prey densities encountered. Bird predation influenced *P. erichsonii* population trends at low insect densities, and possibly at higher densities. Numerical responses were suggested for all except those of sporadic occurrence. The impact of such predation, particularly on the adult stage, suggests that birds may be a significant factor in the regulation of sawfly populations up to moderate infestation levels (Buckner and Turnock 1965). In Kiso District, central Japan, some of birds responded quickly as foraging activity in newly infested stand (Ishida and Tachibana 1986). Furthermore, fifth instar larvae may be eaten by frogs when they drop to the ground. Two species of frogs, *Rana sylvatica* and *R. pipiens*, consumed 210 and 110 larvae per frog per day, respectively (Buckner 1952, cited in Drooz 1960; Turnock 1960).

#### Host suitability

Both of variations in the long shoot and foliage production of the host tree were important biotic factors affecting *P. erichsonii* populations (Turnock 1960). A limited number of new long shoots affected the number of eggs deposited (Drooz 1960; Turnock 1960) and small amount of spur shoot needles limited the number of larvae that have completed development (Turnock 1960). Food quality as well as food quantity can influence development of folivorous insects (e.g. Clancy et al. 2004; Haukioja 2003; Hochuli 1996). However, no detailed studies for *P. erichsonii* and tree defense of its host are available.

#### Abiotic mortality factors

Several reports concerned abiotic (physical) factors influencing *P. erichsonii* development and survival. Lejeune et al.(1955) described the relationship between flooding and survivorship of *P. erichsonii* cocoons. Overwintered eonymph stage was highly resistant to flooding in the spring. Approximately 50 days of continuous flooding were needed to produce 100% mortality. However, 50% died by being submerged for 2 weeks. Two critical periods, fresh spun cocoons in the fall and a post-diapause stage in the spring, were suffered high mortality. Oxygen consumption was highest in those periods, which appeared to be directly related to mortality in the cocoon. One hundred percent mortality is produced by submerged newly spun cocoons for period of 14-28 days, pronymphs for 21 days, pupae for 7 days, and unemerged adults for 2 days. Drouin et al. (1968) similarly presented larval and cocoon stage of *P. erichsonii* was susceptible to mortality from flooding. The development and the decline of *P. erichsonii* outbreaks are likely to be influenced by precipitation cycles. However, because flooding may cause direct damage to trees and parasitoids in the soil, the use of flooding in order to control *P. erichsonii* populations would only be feasible in the rare instances where topographic features permit easy regulation of water levels (Muldrew 1956). High temperature may also influence development and survival to the adult stage: heat discomfort to *P. erichsonii* adults cause such as loss of coordination or violent activity (Turnock 1960). When the temperature raised to 40 °C, the feeding larvae and adults were suffered appreciable mortality (Ives 1968). Prolonged hot and dry weather can also cause desiccation of the larval inside it cocoon (Graham 1956b). The effect of the sun's rays on fifth instar was quite striking in dry site. The larvae attempted to crawl up the tree stems and many larvae died and baked hard on the tree trunks (Drooz 1960). Furthermore, strong winds and heavy rain may cause mortality during feeding larvae (Graham 1956b) and egg stage (Turnock 1960).

From the literature reviews above, the *P. erichsonii* has been the subject of intensive study by many researchers. The history of outbreaks and factors affecting their population dynamics in North America were intensively reviewed (e.g. Drouin et al. 1968; Ives 1976; Nairn et al. 1962; Reeks 1954; Turnock 1960). Several factors responsible for population outbreaks of *P. erichsonii* included insect predators and parasites (Muldrew 1955), pathogens (Smirnof 1968), and small mammals (Buckner 1956) that have been identified as natural enemies of *P. erichsonii*. However, no factors have been reported as acting a density-dependent manner to regulate the population outbreaks of this species. On the contrary,

the foliar properties have been shown to influence the population dynamics of herbivores but no information appears to be available on *P. erichsonii* and its interaction with its host tree. Although, natural enemies have received much attention, this dissertation may not have the same parasitoids, pathogens, and small mammals that occur in the native area of *P. erichsonii*. Therefore, both field and laboratory conditions were decided for studying the insect population trend and assessments of the biotic mortality factors. The work on parasitoids, pathogens, small mammals, and host tree in response to insect defoliation will be compiled in this study. Additionally, this dissertation describes the method used to evaluate the number of newly spun cocoons and predation by small mammals with high precision.

### **Research sites and insect outbreak history**

This study was carried out in the University of Tokyo Hokkaido Forest (UTHF; 142° 18' - 40' E, 43° 10' - 20' N) located in central Hokkaido, Japan (Fig. 1-3). In the UTHF territory (22,733 ha of forest area), small patches of larch plantations (158.48 ha in total (0.7% of area)) are scattered (The University of Tokyo Forest 2012a). The study was conducted at eight stands (sites 1-8) of larch plantation of *Larix kaempferi* (= *L. leptolepis*) with one exception of site 4, in which *L. gmelinii* var. *japonica* (= *L. dahurica*) and their hybrid (*L. gmelinii* var. *japonica* x *L. kaempferi*) were planted. The elevation of these sites ranged from 294 to 660 m ASL. The distance between two of the eight sites ranged from 2.6 to 19.4 km. Small mammal trapping was conducted at two stands of *Picea glehnii* plantation and two stands of natural boreal forest that consisted of broadleaf and coniferous trees (410-680 m ASL): LP, a plantation at lower elevation (420 m ASL); HP, a plantation at higher elevation (550 m ASL); LN, a natural forest at lower elevation (410 m ASL); HN, a natural forest at higher elevation (680 m ASL). The distance between two of the four trapping sites ranged from 50 m (LP-LN) to 7.2 km (LN-HN). The distance from the trapping site to the cocoon sampling site ranged from 2.9 to 19.8 km. At Rokugo (43° 18.1' N, 142° 31.3' E, 315 m ASL), the weather station of the Japan Meteorological Agency nearest to the study area, the average maximum and minimum temperatures and annual precipitation were 32.7°C, -26.0°C, and 1,162 mm, respectively, for the 4 years from 2009 to 2012 (Japan Meteorological Agency 2013). The period of snow

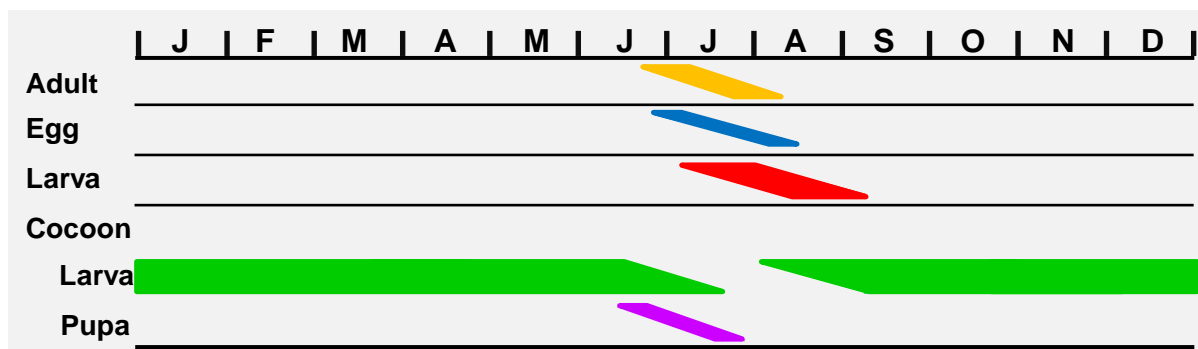
cover usually starts at the end of November and lasts until the beginning of April (The University of Tokyo Forest 2012b).

Larch species are not native to Hokkaido Island. Some species of larches, such as *L. kaempferi*, *L. gmelinii* var. *japonica*, and *L. olgensis*, have been introduced. Among these *L. kaempferi* and *L. gmelinii* var. *japonica* x *kaempferi* have been planted in a large area. Therefore, *P. erichsonii* is also exotic to Hokkaido Island. Larches have two different types of shoots, spur shoots and long shoots. The spur shoots are short woody shoots, typical of flush growth, that bear needles in dense clusters (20-40 needles). The long shoots (both terminal and lateral) are rapidly growing shoots and non-woody green shoots, which bear individual needles in a spiral pattern.

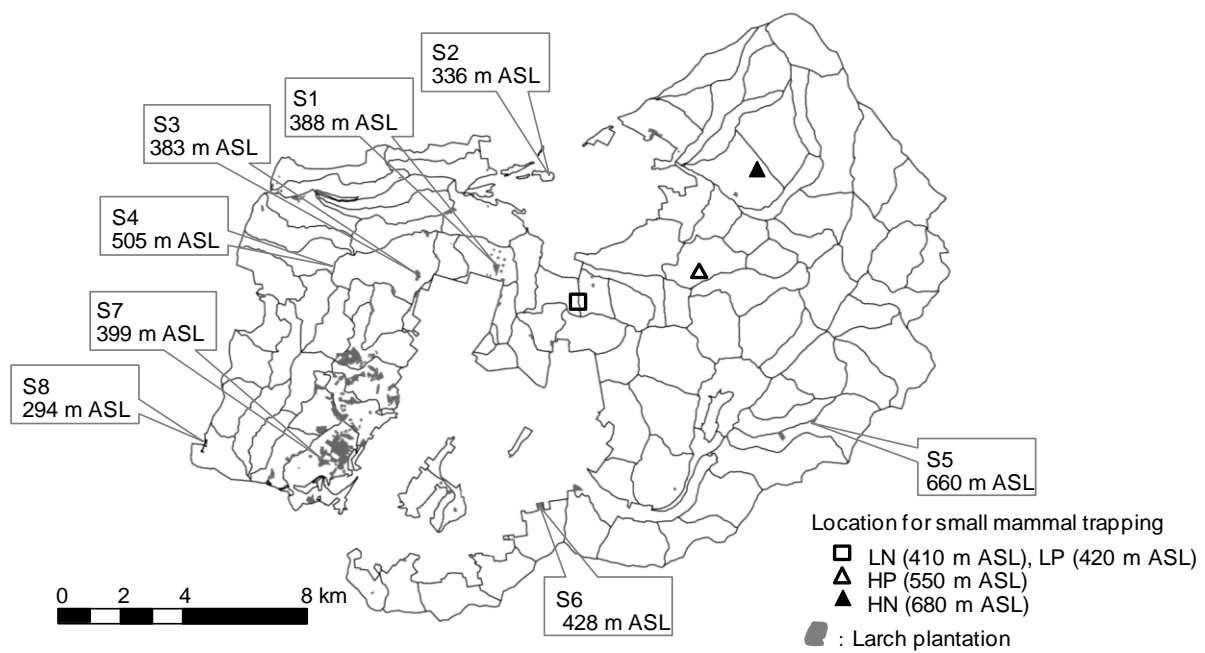
Past population outbreaks of *P. erichsonii* in Hokkaido occurred from 1977 through 1986 (Higashiura 1990). The relevant population outbreaks of *P. erichsonii* were summarized by the Hokkaido Prefecture (<http://www.pref.hokkaido.lg.jp/sr/srs/grp/karamatuharaakahabachi.pdf>). The population outbreak of *P. erichsonii* was found in the Oshima Peninsula on the southwestern part of Hokkaido Island during the years 1995-2002 and has started to spread to the northeast, beginning in 2004. In Furano City, where the UTHF is located, conspicuous defoliation by *P. erichsonii* has been recorded since 2008. In the UTHF, defoliation by *P. erichsonii* became conspicuous at sites 6 and 7 in 2009. No population outbreaks of other folivores have occurred on larch trees in the UTHF in five years (2008-2012) (Panisara Pinkantayong, personal observation). Hence, a delayed induced response to insect defoliation should have been minimal in 2009.



**Figure 1-1** Some aspects in the life cycle of the larch sawfly, *Pristiphora erichsonii*. **A**, Adult female; **B**, eggs in tissues of new long shoots; **C**, Young larvae feed gregariously on needles; and **D**, After maturing, larvae drop, enter into the soil and spin cocoon for overwintering



**Figure 1-2** Generalized life cycle of the larch sawfly, *Pristiphora erichsonii* in the University of Tokyo  
Hokkaido Forest, central Hokkaido, Japan



**Figure 1-3** Locations of eight research sites for studying insect population density and four stands for small mammal trapping in the University of Tokyo Hokkaido Forest, central Hokkaido, Japan

## CHAPTER 2: POPULATION DENSITY

### 2.1. Introduction

This study presents detailed information on *P. erichsonii* population as follows:

#### 1) Evaluation of defoliation intensity

In the previous studies mostly carried out in N. America, defoliation by *P. erichsonii* was commonly surveyed from the ground and aerial. Ground surveys are based on estimates of defoliation classes whereas aerial surveys are based on discernible color ratings (Nairn and Prentice 1960). However, in this study, the insect defoliation intensity of four successive years (2009-2012) was evaluated for each individual tree by comparing canopy photos taken in June and October of the same year. Both full-color and near-infrared (NIR) photos were used for improving accuracy of evaluation (Chapman 2007).

#### 2) Estimation of cocoon abundance and small mammal predation by a hierarchical Bayesian model

The cocoon stage of *P. erichsonii* has received more detailed investigation than adult, egg, and free-living larval stages for several reasons. Basically, cocoon stage is thought to be important in determining population dynamics of sawflies, because *P. erichsonii* spend approximately 10 months inside a cocoon in the soil and because sawfly cocoons are vulnerable to small mammal predation (Graham 1928; Hanski and Parviainen 1985; Holling 1959). Furthermore, cocoons are easy to sample, incubate, and manipulate (Ives and Turnock 1959; Lejeune 1955). Several methods have been used to estimate the predation of *P. erichsonii* cocoons by small mammals, which include cocoon planting, cocoon sampling, and protection of cocoons from small-mammal predation in forests (Buckner 1959). However, these methods had problems in precision of the estimates. Cocoon planting changes the local density and distribution pattern of cocoons, which strongly influence the response of small mammals. Cocoon sampling cannot provide a precise estimate of the number of newly spun cocoons or of cocoons preyed on by small mammals. This issue exists because sawflies are exposed to small mammal predation during cocoon stage, with the timings of cocoon spinning and adult emergence greatly varying within population (Turnock 1960). Furthermore, the cocoon of the current generation cannot be distinguished from older ones by the naked eye (Ives 1976). Molecular analysis could be used to determine the freshness of the cocoons. However, it would be expensive and time consuming to conduct this technique on thousands of empty cocoons. Therefore, in this



study, a hierarchical Bayesian model was developed to estimate the number of newly spun cocoons and predation rate by small mammals from annual one-time soil sampling datasets. The model was applied to the cocoon dataset of *P. erichsonii*, which was annually sampled at each site for 4 years (2009-2012).

### 3) Frass-drop studies

Several researchers have used frass-drop samples as an indirect measurement of the density of forest insect populations (Fischbacher et al. 1998; Green and deFreitas 1955; Higashiura 1987; Hijii et al. 2001; Kamata 2000) and used as a method for investigation of the amount of damage to host plants caused by insect feeding (Morris 1960; Simandl 1989; Zandt 1994). In this study, the seasonal incidence of insect defoliation and foliage consumption were estimated using the frass-drop samples. Furthermore, previous study have suggested the number of larvae can be estimated by fallen head capsules (Higashiura 1987). Head capsules obtained from the frass drop trapping in this study were used to measure the larval population density of each instar and to construct a life table of *P. erichsonii* populations.

## 2.2. Materials and Methods

### 2.2.1. Evaluation of defoliation intensity by canopy photos

In October 2009, three canopy photos were taken at eight research sites (Fig. 1-3) with a fish-eye lens (Sigma, 4.5 mm F2.8 EX DC circular fisheye HSM). At each site, the three photos were taken at three points set linearly at 5-m intervals. A total of 318 trees were recognized in the photos and individually numbered. The number of individual larch tree, of which defoliation intensity was estimated, ranged 22-72 depending on the site.

Since 2010, canopy photos have been taken monthly from June to October at these same points in both full color and the near-infrared (NIR) band (Fig. 2-1). Both types of canopy photos were transformed to panorama photos and referred to the individual tree numbering (Fig. 2-2). The insect defoliation intensity of each numbered tree was evaluated at 10% intervals (0, 10, ..., 100%) by comparing photos that were taken in June and October of the same year. This approach was based on the timing of the larval period of *P. erichsonii* (mid-July to mid-September in Hokkaido) and the timing of natural needle fall (early to mid-November). To improve the accuracy of the estimates, both the full-color and the NIR photos were used for the evaluation of defoliation (Chapman 2007). Because the defoliation intensity study started in

October 2009, the 2009 defoliation intensity was evaluated by comparing the October-2009 and June-2010 photos.

### **2.2.2. Seasonal incidence of insect defoliation by fallen frass**

In 2010 (1-30 August), 2011 (23 July-8 September), and 2012 (18 July-12 September), five traps with an opening 1 m<sup>2</sup>, consisted of 1x1 m fine-mesh cloth stretched across wooden stakes, were placed about 1 m above ground beneath each tree crown in each research site. The bottom of trap was fixed on small wooden stake at center for preventing samples collected blown out by wind. Trap content was generally collected once a week until finishing larval feeding period. The intervals varied from 4 to 9 days according to weather condition. The trap contents then dried up at 60 °C with electric oven for few days. The other debris such as twigs, branches, barks and dead insects were removed before determining dry weight of frass. Total dry weight of frass for each trap was determined by the sum of the dry weight at each collecting date each year. Date of 50% cumulative falling frass was also determined by interpolation using data of the last collecting date below 50% and those of the first collecting date above 50%.

### **2.2.3. Estimated larval density using fallen head capsules**

Density of head capsules of *P. erichsonii* larvae in 2011 was estimated from sampled frass collection by the litter trap method (see Subsection 2.2.2). In 2012, the other types of funnel cloth traps (opening 14 cm in diameter) were used. Three funnel cloth traps were deployed beneath the canopy of each same sample tree as each of the litter traps from 18 July to 12 September, 2012. Treatment of the trap contents was same as the methods mentioned in the section 2.2.2. Head capsules of *P. erichsonii* larvae were collected and classified into each of four instars by their color (Fig 2-3). Color of head capsule in the first instar was transparent light brown. After molting, the larva is in the second instar and head capsule color was light brown. From third to fourth instar, the head capsule color was dark brown. Finally, the head capsule was changed to black in the fourth instar. Eight hundred and seventy head capsules were arbitrary selected and were measured the distance between lateral sides of head capsule margins under a stereo binocular microscope fitted with a calibrated ocular micrometer eyepiece. Histogram of the size was plotted for each instar.

#### **2.2.4. Estimation of cocoon abundance and small mammal predation by a hierarchical Bayesian model**

In mid-October from 2009 to 2012, ten top soil samples (20 x 20 cm each with approximately 15 cm depth) were collected, because it has been reported that *P. erichsonii* mature larvae enter the soil and spin cocoons at depths shallower than 7 cm (Higashiura and Suzuki 1981). The topsoil samples were collected from each site, according to a specific methodology. In 2009, five sample trees were selected arbitrary in each site, depending on the level of defoliation intensity. Trees that had been subjected to the least (<30% of defoliation intensity), intermediate (30-60%), and greatest (>60%) levels of defoliation intensity were selected as the sample trees (27, 5, and 6 individuals from the least, intermediate, and greatest levels, respectively). The sample trees were then used throughout this study. The distance between the two nearest sample trees was approximately 4-8 m. Two topsoil samples were collected from under the canopy of each of the sample trees. The distance between any two samples below one tree was approximately 1-5 m. When sampling the soil, the sites of former sample collections were avoided because the abundance of old cocoons would be underestimated. The collected soils were then transferred to a nursery of the UTHF. Cocoons of *P. erichsonii* were then manually collected by sieving and hand sorting. The cocoons were separated into three categories: unopened healthy-looking cocoons with a healthy larva or a parasitized larva (hereafter referred to as “unopened cocoons”), empty cocoons caused by small-mammal predation, and empty cocoons caused by something other than small-mammal predation. For cocoons with a hole, the cause was judged on the basis of the appearance of the hole (Krause and Raffa 1996): 1) a smooth and spherical exit hole at the end of the cocoon indicated the normal emergence of a *P. erichsonii* adult, 2) a small and slightly jagged spherical exit hole near the end of the cocoon indicated a parasitic wasp, 3) a very small non-spherical exit hole at the end of the cocoon indicated a parasitic fly, and 4) a jagged or shredded opening of variable size indicated small-mammal predation (Fig. 2-4) (Buckner 1956; Dahlsten 1967; Ives 1976). Because predation by small mammals occurs during the period immediately after the cocoon is spun until adult emergence, the cocoons preyed on by small mammals from the soil samples included cocoons of both the current generation and previous ones, which were indistinguishable from each other by the naked eye (Ives 1976; Ives and Turnock 1959). Empty cocoons caused by something

other than small-mammal predation included the normal emergence of a *P. erichsonii* adult, parasitoid emergence, and those killed by diseases of previous generations.

A cocoon dynamics model based on a Bayesian approach was developed to estimate: the abundance of the cocoons in the current generation before small-mammal predation, predation rate during the period from cocoon spinning to sampling in October (hereafter referred to as “predation rate before October sampling”), and predation rate from sampling in October to adult emergence in the following summer (hereafter referred to as “predation rate after October sampling”) (Fig. 2-5). Small mammals prey on unopened cocoons without discriminating between healthy cocoons and cocoons parasitized by parasitoids (Buckner 1956; Hardy 1939). Thus, an identical parameter was used for each of the two predation rates before and after October sampling. In addition, there is no evidence to suggest that small mammal feed on a whole cocoon (personal communication). The hierarchical model consists of data models and process models. The data models relate to the observed number of cocoons in sample  $j$  at site  $i$  in year  $t$  and to the expected number of cocoons in the same sample ( $\lambda_{I,i,j,t}$ ,  $\lambda_{M,i,j,t}$  and  $\lambda_{H,i,j,t}$ ), in which the observed cocoons are unopened cocoons ( $I_{i,j,t}$ ), empty cocoons due to small-mammal predation ( $M_{i,j,t}$ ), and empty cocoons caused by something other than small-mammal predation ( $H_{i,j,t}$ ). Poisson models were used because the number of cocoons is count data, whereby:

$$I_{i,j,t} \sim \text{Pois}(\lambda_{I,i,j,t})$$

$$M_{i,j,t} \sim \text{Pois}(\lambda_{M,i,j,t})$$

$$H_{i,j,t} \sim \text{Pois}(\lambda_{H,i,j,t})$$

The process models describe how the numbers of cocoons in each category in each year are related to each other.  $\lambda_{I,i,j,t}$  is the number of cocoons spun in the summer of year  $t$  and not preyed on by small mammals by the time of sampling in October.  $\lambda_{M,i,j,t}$  is the sum of the following: cocoons spun in the summer of year  $t$  but subjected to small-mammal predation by the October sampling of year  $t$ , cocoons spun in the summer of year  $t-1$  but subjected to small-mammal predation to the summer of year  $t$ , and cocoons spun in the summer before year  $t-1$  but subjected to small-mammal predation in the summer of the year before  $t-1$ .  $\lambda_{H,i,j,t}$  is the sum of empty cocoons caused by something other than small-mammal predation, which were spun before year  $t$ . The model formulae are as follows:

$$\lambda_{L,i,j,t} = (1 - \theta_{i,j,t}) N_{i,j,t},$$

$$\lambda_{M,i,j,t} = \theta_{i,j,t} N_{i,j,t} + \varphi(\kappa_{i,j,t} I'_{i,j,t-1} + M'_{i,j,t-1}),$$

$$\lambda_{H,i,j,t} = \varphi(H'_{i,j,t-1} + (1 - \kappa_{i,j,t}) I'_{i,j,t-1}),$$

where  $\theta_{i,j,t}$  and  $\kappa_{i,j,t}$  are the predation rate before October sampling and the predation rate after October sampling in sample  $j$  at site  $i$  in year  $t$ , respectively.  $N_{i,j,t}$  is the number of cocoons spun in year  $t$  in sample  $j$  at site  $i$  in year  $t$ .  $\varphi$  is the annual remaining rate of empty cocoons, which ranged from 0 to 1. A prior distribution of  $\varphi$  was assumed to be a uniform distribution, with  $U(0,1)$ .  $I'_{i,j,t-1}$ ,  $M'_{i,j,t-1}$  and  $H'_{i,j,t-1}$  are latent variables for the number of unopened cocoons, opened cocoons (due to small-mammal predation), and empty cocoons (caused by factors other than small-mammal predation), respectively, from the previous year ( $t-1$ ) that were already present in the soil samples of the current year ( $t$ ). I used informative prior distributions for the parameters, and gamma distributions with the same mean and variance as the observed distributions of  $I_{i,j,t-1}$ ,  $M_{i,j,t-1}$  and  $H_{i,j,t-1}$ . Of note, this is an important component of the Bayesian model because it was unable to determine how many cocoons from previous years remained in the soil samples of the current year directly, which represented an important limitation. This approach does not lead to duplicated use of data because  $I'_{i,j,t-1}$ ,  $M'_{i,j,t-1}$  and  $H'_{i,j,t-1}$  are used to estimate the number of cocoons in current year ( $\lambda_{L,i,j,t}$ ,  $\lambda_{M,i,j,t}$  and  $\lambda_{H,i,j,t}$ ) but not in the previous year ( $\lambda_{L,i,j,t-1}$ ,  $\lambda_{M,i,j,t-1}$  and  $\lambda_{H,i,j,t-1}$ ).  $\theta_{i,j,t}$  and  $\kappa_{i,j,t}$  were assumed to follow beta distributions  $\text{Be}(\alpha=k_{\theta}p_{\theta,i,t}, \beta=k_{\theta}(1 - p_{\theta,i,t}))$  and  $\text{Be}(\alpha=k_{\kappa}p_{\kappa,i,t}, \beta=k_{\kappa}(1 - p_{\kappa,i,t}))$ , respectively, where  $p_{\theta,i,t}$  and  $p_{\kappa,i,t}$  are the mean predation rate before October sampling and the mean predation rate after October sampling at site  $j$  in year  $t$ , respectively.  $k_{\theta}$  and  $k_{\kappa}$  are parameters that determine the dispersion of the distribution, and an informative  $\text{Gamma}(\alpha=1, \beta=1)$  prior, where  $\alpha$  and  $\beta$  was assumed to be the shape and rate parameters. Because  $N_{i,j,t}$  is a positive continuous variable, it is assumed to be distributed following a gamma distribution,  $\text{Gamma}(\alpha=N_{i,t}/s, \beta=1/s)$ , where  $N_{i,t}$  is the mean number of cocoons of the generation in year  $t$  per sample at site  $i$  in year  $t$ . Non-informative priors are used for hyperparameters. For instance, a uniform distribution,  $U(0, 1)$  is used for parameters between 0 and 1,  $\varphi$ ,  $p_{\theta,i,t}$  and  $p_{\kappa,i,t}$ . In comparison, an inverse gamma distribution,  $\text{InvGamma}(\alpha=0.001, \beta=0.001)$ , is used for parameters  $>0, s$ ,  $N_{i,t}$ ,  $k_{\theta}$  and  $k_{\kappa}$ , where  $\alpha$  and  $\beta$  are the shape and scale parameters, respectively. The total

predation rate throughout the cocoon period (hereafter referred to as the “total predation rate”) ( $\rho$ ) was obtained by the following equation:

$$\rho_{i,t} = 1 - (1 - \theta_{i,t})(1 - \kappa_{i,t}).$$

The Random-walk Metropolis-Hasting algorithm (Hastings 1970; Metropolis et al. 1953), which is Markov chain Monte Carlo (MCMC) method, was used to sample from the posterior distributions of model parameters. The MCMC sampling algorithm was written in C. Three MCMC chains for  $5 \times 10^5$  iterations, with a burn in of  $2 \times 10^5$  iterations were run. The MCMC samples were thinned to one every 100th samples, to reduce autocorrelation and due to computational limitations. Convergence of the MCMC was checked by Gelman-Rubin statistic. The 95% credible intervals (CIs) of the posterior distribution of parameters were calculated from the 95% highest posterior probabilities by using the ‘HPDinterval’ function from the ‘coda’ library (Plummer et al. 2012) in R ver. 2.15.3 (R Development Core Team 2013).

To assess the fit of model, the posterior predictive  $P$ -value was computed using deviance as a test quantity (Gelman et al. 2004), where an extreme  $P$ -value (smaller than 0.05 or greater than 0.95) indicates a large discrepancy between the actual data and the model.

#### **2.2.5. Adult and egg density**

In October of each year, unopened healthy-looking cocoons that were collected from top soil samples (cf. subsection 2.2.4) were transferred into non-woven nylon bag and incubated in planters, with sterilized vermiculite for overwintering. After overwintered, it was individually moved to a plastic vial with moist tissue for checking an emergence of living inhabitant. Emerged *P. erichsonii* adult was identified sex by presence/or absence of an ovipositor. Then, adult female density per unit area was determined by considering predation rates by small mammals after October sampling ( $\kappa$ ). The number of eggs deposited by adult female was also calculated by multiplying adult female density and the average fecundity of *P. erichsonii* females in Hokkaido (38.1 eggs per female) (Higashiura 1988).

### **2.3. Results**

#### **2.3.1. Evaluation of defoliation intensity by canopy photos**

In 2009, the number of lightly defoliated larch trees was greatest among the four years. However, the defoliation intensity varied greatly among the eight sites. Most research sites received light defoliation

(1.7-7.8% of average defoliation intensity), with two exceptions of sites 6 (Average=70%, median=80 %) and site 7 (Average=87.9%, median=90%). In 2010, most larch trees were entirely defoliated by *P. erichsonii* larvae. The greatest defoliation intensity was observed at site 7 (median=100). In 2011, the number of severely defoliated larch trees continued to increase at most research sites with two exceptions of sites 7 and 8, where median value slightly decreased from 2010 to 2011. The number of severely defoliated larch trees decreased greatly in 2012. However, there are still many severely defoliated trees at sites 6 (median=20%) and 7 (median=70%) (Fig. 2-6). The Spearman's rank correlation coefficient between two years of defoliation intensity of individual trees was determined for each site each year (Table 2-1). Significant coefficients were found only in the combination of years with severe defoliation. All significant coefficients were positive indicating that defoliation intensity significantly depended on individual tree.

### **2.3.2. Seasonal incidence of insect defoliation by fallen frass**

Total frass weight increased from 2010 to 2011 with one exception of site 8, in which the frass weight slightly declined. In 2012, the frass weight was smallest among the three years with one exception of site 7, in which the frass production continued to increase. The frass production was greatest at site 2 in 2011. The frass production was small at sites 4 and 7 compared to other sites. The total frass weight and defoliation intensity changed similarly in all sites with one exception of site 7. However, significant positive correlation were found ( $r=0.84$ ,  $P<0.01$ ,  $n=24$ ) (Fig. 2-7).

Seasonal incidence of frass yield showed similar pattern among eight research sites but varied greatly among years. In general, frass was collected from mid-July to mid-September. In 2010, fallen frass peaked earlier than other years at all research sites. Namely, 50% of cumulative falling frass was observed at 217-222 Julian date. In 2010, the greatest mean frass yield was observed at site 2 (196.2 g/m<sup>2</sup>) and the lowest at site 4 (51.7 g/m<sup>2</sup>). In 2011, the greatest mean frass yield (231.0 g/m<sup>2</sup>) was also observed in the site 2 and smallest (82.5 g/m<sup>2</sup>) in the site 4. Fifty percent of cumulative falling frass date in 2011 ranged at 220-226. The mean frass yield greatly decreased in 2012 in most research sites except at site 7. The date of 50% cumulative falling frass in 2012 ranged at 221-231 (Fig. 2-8).

### 2.3.3. Estimated larval density using fallen head capsules

The histogram of the larval head capsule widths shows that the size ranged from 0.18 to 0.52 mm (Fig. 2-9). The size of each instar was 0.17-0.26 mm for the first instar ( $n=183$ ); 0.24-0.32 mm for the second instar ( $n=66$ ); 0.31-0.43 mm for the third instar ( $n=469$ ) and 0.44-0.52 mm for the fourth instar ( $n=152$ ). Overlap between adjacent instars occurred for the first three instars.

The density of each instar based on head capsules widths shown in Table 2-2 for generation 2011 and 2012. Larval density was higher in 2011 with one exception of site 7. The estimated density sometimes increased with development. The estimated density had significant positive relationship both with defoliation intensity ( $r=0.80$ ,  $P=0.01$ ,  $n=16$ ) and with total frass weight ( $r=0.93$ ,  $P<0.01$ ,  $n=16$ ).

### 2.3.4. Estimation of cocoon abundance and small mammal predation by a hierarchical Bayesian model

The abundance of unopened cocoons ( $I$ ) in the field peaked in 2010 or 2011, depending on the site, and decreased in six sites in 2012. The observed abundance of  $M$  and  $H$ , which was only influenced by the current generation, decreased greatly at many sites in 2012 (Table 2-3). The model estimated the annual remaining rate of cocoons ( $\phi$ ) at 0.743 (95% CI, 0.722 - 0.768; Table 2-4). The estimated abundance of  $N$  fluctuated in a similar manner to the observed  $I$  in the model. The modeled predation rate before October sampling ( $\theta$ ) was prone to increase with year. There was no constant tendency in the rates of predation by small mammals between the period before and after October sampling ( $\theta$  and  $\kappa$ ). Total predation rate ( $\rho$ ) was small in 2009, but was higher at sites 6 and 7 compared to the other sites. Mortality increased during 2010 and 2011 (Table 2-4). The posterior predictive  $P$ -values indicate that the model adequately explained the collected field data (0.54 for  $I$ , 0.48 for  $M$  and 0.07 for  $H$ ).

Figure 2-10 shows relationship between density of instar IV and that of  $N$  in generations 2011 and 2012. No significant correlation was observed between them ( $r=0.43$ ,  $P>0.05$ ,  $n=16$ ). However, significant positive correlation was found if two data with the highest density of instar IV were excluded ( $r=0.85$ ,  $P<0.01$ ,  $n=14$ ). These two data shown high density of instar IV but low density of newly spun cocoons indicating that many larvae failed spinning cocoons.

### 2.3.5. Adult and egg density



Table 2-5 shows estimated densities of adults and females of generations from 2009 to 2011 and estimated adult density of a generation 2012 with considering small-mammal predation after sampling. The densities were greatest in generation 2009 at sites 4-7, in generation 2010 at sites 1-3, and in generation 2011 at site 8. The densities decreased greatly in the generation 2012 though small-mammal predation after sampling was not taken into consideration. Proportion of females was high with small variation by year (0.93-1). All adults were females in generation 2012.

A simplified format of life table is summarized in Appendix 1 by adding numbers of eggs estimated from the female abundance.

## **2.4. Discussion**

The number of head capsules from canopy is a good indicator to estimate larval density. However, one individual produces one head capsule during molting so the necessary number of traps together with their size and layout are especially an important especially during low density periods and for gregarious species (Kamata et al. 1994). Larval density should decrease with development due to mortality whereas the densities estimated using head capsules sometimes increased with instar (Table 2-2). Size, number, and layout of litter traps should be optimized for estimating larval density using head capsules in future studies. Furthermore, *P. erichsonii* lays the eggs in long shoots that is distributed near terminal of each branch more than near a trunk. However, litter traps were set near tree trunks (1-2 m apart from tree trunks) so that young instars were likely to be underestimated. Dispersal distance was also likely to differ among instars. Many studies on seed dispersal were reported that intraspecific morphological variation affected dispersion potential (Thiede and Augspurger 1996; Winson and Traveset 2000). Fruit shape, area and weight were related to flight duration of fruits of *Fraxinus mandshurica* var. *japonica* (Goto et al. 2005). It is also likely that a kernel function of dispersal distance of molded head capsules differs among instars.

Several reports suggested fallen frass reflected the feeding activity of forest defoliating insects so that frass trapping provided good estimates of larval density (Drooz 1960; Higashiura 1987; Kamata et al. 1994). Both values of defoliation intensity and of fallen frass weight were closely related to each other so they changed in similar manner with one exception of site 7. Defoliation intensity and total frass weight had a peaked in 2011 in all research sites, with two exceptions of sites 7 and 8 (Fig 2-7). At site 7,

defoliation intensity peaked in 2010 but frass weight did in 2012. At site 8, both defoliation intensity and frass weight peaked in 2010. However, a significant positive correlation was found between the two values.

From a viewpoint of temporal dynamics, the population density in each research site was classified into three groups. Group 1, including sites 1-5 and 8, severe defoliation continued only two years in 2010 and 2011 by taking account to pulse or cyclic type with unstable high equilibrium point. On the contrary, Group 2, site 7, severe defoliation continued throughout the four years by taking account to sustained type of eruption. Group 3, site 6, severe defoliation continued three years from 2009 to 2011, which was intermediate between Groups 1 and 2. Spatially, in 2009, a conspicuous defoliation by *P. erichsonii* was observed in small areas around sites 6 and 7. During 2010-2011, the severely defoliated area was found extensively in the UTHF and its surroundings. Severe defoliation shrank to small areas around site 7 in 2012. Hence, site 7 can be regarded as an epicenter of the population outbreaks (Fig 1-3). According to Ryo Hotta (personal communications), the density was decreased slightly in 2013 with conspicuous defoliation in and around site 7 but increased again in most of locations in 2014: Conspicuous defoliation was observed in all the eight research sites with two exceptions of sites 4 and 5, severe defoliation at sites 2, 3, and 6, and moderate defoliation at sites 1, 7, and 8. The resource concentration hypothesis (Root 1973), which holds that insect herbivores are more likely to locate and to remain on dense stands, is likely to explain the site-dependent characteristics of *P. erichsonii* population outbreaks. A large cluster of larch plantations were established around site 7 (Fig. 1-3).

At site 7, seasonal incidence of fallen frass peaked at the first collecting date in 2010. It peaked earlier in 2010 than the other years at all research sites. Warmer climate in 2010 was the most likely cause of the phenomenon. High larval density at site 7 in 2010 was also likely related to the phenomenon because the total frass weight was smaller than those expected from defoliation intensity at site 7 in 2010. Timing of setting litter traps may have been late compared to timing of insect feeding. As a result, total frass weight was very likely to be underestimated. The same trend was found in cumulative curves of fallen frass at sites 6 and 8 in the same year (2010). Hence, the total frass weight may also have been underestimated at sites 6 and 8 in 2010 though conspicuous inconsistency was not found between defoliation intensity and total frass weight. Total fallen frass was influenced by larval density when the density was not high enough to cause complete defoliation. Actually a significant positive correlation was

found between density of instar IV estimated using head capsules and total fallen frass though the data of head capsule were available only in 2011 and 2012. However, foliage biomass in canopy would be determinant of the total frass weight when they receive complete defoliation. Although total frass weight was likely underestimated at sites 6-8 in 2010, the total frass weight at site 2 in 2011 was greatest followed by site 5 of the same year indicating that the canopy foliage biomass was greatest at site 2 among the eight sites. Defoliation intensity was greatest at site 7 of 2010 (Fig. 2-6). However, at site 7, canopy was not closed yet after recent thinning. Actually, the total frass weight at site 7 was smaller than 150 g/m<sup>2</sup> in 2011 and 2012 despite of intense defoliation (Fig. 2-8).

Density of instar IV in 2011 was estimated using head capsules and recorded maximum at site 5 followed by site 2 (Table 2-2). Both sites received complete defoliation in 2011 (Fig. 2-6). However, sizes of individual trees differed to a great extent between the two sites. The tree height was measured approximately 15 m above ground level at site 5 whereas approximately 30 m at site 2 (Panisara Pinkantayong, personal observation). Small tree height at site 5 was probably due to shallow topsoil. In August 1983, before larch saplings were planted, the top soil was scarified to a depth of 20 cm from the surface by bulldozers (Owari et al. 2011).

Significant positive correlations were found in 18 combinations of two years of defoliation intensity at an individual tree level assessed by canopy photos (Table 2-1). Only one significant negative correlation was found at site 4 between 2009 and 2010. These results suggest that defoliation intensity depends on a character of each individual tree. The other possible explanation is that heavily infested trees would be deposited many eggs in the following year if mobility of *P. erichsonii* is low. However, the latter is unlikely because of following reasons: Density of instar IV at site 5 was greatest in 2011 among eight sites in spite of smallest cocoon density in 2010. The total frass weight at site 5 was third greatest in 2011 whereas smallest cocoon density in the same year suggest low survivorship between late free-living larvae and cocoons. The topsoil was still shallow at site 5 during our study (data were not shown). The failure of most larvae to spin cocoons at this site was probably due to the shallow topsoil layer. High density of *P. erichsonii* larvae at site 5 in 2011 was very likely derived from females that had immigrated from neighboring larch plantations without scarification. Mobility of *P. erichsonii* is so high that the latter hypothesis is very unlikely.

In this study, a hierarchical model was developed to estimate the number of newly spun cocoons and the number of cocoons preyed on by small mammals from annual cocoon sampling in October over a four-year period (2009-2012). This model was based on the assumption that the remaining rate of empty cocoons was constant among years and locations. I believe that this assumption is reasonable because the decomposition rate of empty cocoons is influenced by certain environmental factors, such as temperature and moisture, including the activity of bacteria. In addition, I assumed that the number of cocoons formed before 2009 were negligible because the value  $H$ , which was influenced by cocoons that had been spun in the previous year or before, was much smaller in 2009 than in 2012, with the exception of sites 6 and 7 (Table 2-3). When the model was applied to a field dataset, this assumption may have acted as a source of error, especially for sites 6 and 7. Actually, the posterior predictive  $P$ -values of  $I$  was marginally significant ( $P=0.07$ ) though all the posterior predictive  $P$ -values of  $I$ ,  $M$ , and  $H$  in this model were between 0.05 and 0.95, which indicate that the model adequately described the data. Furthermore, the modelling approach presented calculates the level of small-mammal predation of *P. erichsonii* cocoons with high precision.

The remaining rate of empty cocoons ( $\phi$ ) was estimated as 0.743 (Table 2-4), which represents 74.3% of empty cocoons remain for 1 year, 55.5% do for 2 years, 40.9% do for 3 years, and 30.4% for 4 years. Actually, the observed  $M$  and  $H$  tended to increase with year (Table 2-3). The estimated abundance of  $N$  (Table 2-4) fluctuated in a similar manner to the observed abundance of  $I$ , however, generally greater than  $I$  (Table 2-3). This seems reasonable and more reliable estimate because  $N$  values did not include cocoons preyed on by small mammals before October sampling ( $\theta$ ).  $N$  continued to increase from 2009 to 2010 and then declined in 2011 with two exceptions of sites 6 and 7. The abundance of  $N$  at sites 6 and 7 was generally higher than other research sites except in the year 2010, in which the cocoon abundance declined in the two sites (Table 2-4) in spite of the severe defoliation (Fig. 2-6). Many larvae were found crawling on tree trunks at the two sites in 2010 due to food shortage on canopy, which would have starved before completing their development. Similar situation was also found at sites 2 and 5 in 2011: namely much smaller numbers of  $N$  than those expected from larval density (Fig. 2-10). These results explain that food shortage occurred at earlier stage of instar V when the larval density was extremely high. As a result, a fewer individuals could survive and spin cocoons at higher larval density, as known as scramble-type

competition. Regarding site 5, shallow topsoil (Owari et al. 2011) was also a likely cause of smaller numbers of cocoons compared to larval density. Many matured larvae were found died before spinning-up cocoon due to difficulty in entering the soil.

A situation of site 4 seemed different, in which defoliation intensity was small as well as the cocoon abundance (*N*) (Table 2-4). In 2010 and 2011, a large number of eggs were estimated to be deposited in the site. However, total frass weight and density of cocoons were small (Fig. 2-8, Table 2-4). One possible cause was a muddy soil at the site. There were many open pools of water at the site. It was reported that flooding in early spring was a major cause of great mortality inside cocoons, which decreased population outbreaks in United States (Lejeune et al. 1955). Muddy soil with anaerobic condition possibly caused uncompleted spinning up the cocoon. However, it is more likely that low *P. erichsonii* density at site 4 depended on host plant species. At site 4, *L. gmelinii* var. *japonica* and hybrid (*L. gmelinii* var. *japonica* x *L. kaempferi*) were planted. The mean number of eggs deposited per female on *L. kaempferi*, *L. gmelinii* var. *japonica*, and hybrid (*L. gmelinii* var. *japonica* x *L. kaempferi*) was 38.1, 32.8, and 22.8, respectively. The number of eggs per female on *L. gmelinii* var. *japonica* and on the hybrid (*L. gmelinii* var. *japonica* x *L. kaempferi*) was 65% and 48%, respectively, of that on *L. kaempferi* (Higashiura 1988). Chemical properties of *L. kaempferi* foliage differed greatly from those of *L. gmelinii* var. *japonica* and of the hybrid (cf. Appendix 3). The latter two were more defensive than the former. *L. kaempferi* was planted at seven sites other than site 4. *L. gmelinii* var. *japonica* and their hybrid (*L. gmelinii* var. *japonica* x *L. kaempferi*) were planted at site 4. The lower density of *P. erichsonii* at site 4 was very likely due to host larch species.

**Table 2-1** Spearman's rank correlation coefficient of associations between two years of defoliation intensities at eight research sites in the University of Tokyo Hokkaido Forest

Site	Year's defoliation					
	DF09 & DF10	DF09 & DF11	DF 09 & DF12	DF 10 & DF11	DF10 & DF12	DF 11 & DF12
1	0.16	0.12	0.06	<b>0.69</b>	0.21	0.17
2	-0.17	-0.25	-0.17	<b>0.81</b>	0.26	0.20
3	-0.12	-0.28	<b>0.50</b>	<b>0.58</b>	0.04	0.05
4	<b>-0.74</b>	-0.11	0.37	<b>0.57</b>	-0.28	-0.17
5	<b>0.28</b>	0.21	<b>0.33</b>	<b>0.64</b>	-0.01	-0.07
6	<b>0.53</b>	<b>0.55</b>	-0.06	<b>0.68</b>	0.14	-0.08
7	<b>0.59</b>	<b>0.58</b>	<b>0.63</b>	<b>0.45</b>	<b>0.37</b>	<b>0.55</b>
8	-0.18	-0.03	-0.17	<b>0.64</b>	0.29	-0.13

Bold: statistical significance ( $P < 0.05$ )

**Table 2-2** Density of four larval stages of *Pristiphora erichsonii* (Hartig) per m<sup>2</sup> using head capsules collected by frass traps in the University of Tokyo Hokkaido Forest during 2011 and 2012

	Site							
	1	2	3	4	5	6	7	8
<b>Generation 2011</b>								
Instar I	1805.0	2734.0	1026.0	258.0	2197.0	676.0	386.0	1044.0
Instar II	1956.0	2941.0	1037.0	327.0	2227.0	709.0	514.0	1209.0
Instar III	1277.0	2218.0	729.0	332.0	1797.0	705.0	505.0	1028.0
Instar IV	850.0	1178.0	515.0	265.0	1329.0	738.0	450.0	677.0
<b>Generation 2012</b>								
Instar I	59.5	326.8	225.5	86.6	51.4	427.5	865.8	296.5
Instar II	108.2	409.1	351.7	72.2	44.6	441.9	953.6	253.2
Instar III	93.8	300.9	245.3	30.7	74.4	434.7	864.6	246.8
Instar IV	34.3	296.5	257.9	9.0	44.6	568.2	827.3	190.5

**Table 2-3** Abundance of unopened cocoons of *Pristiphora erichsonii* (Hartig) (*I*), empty cocoons preyed on by small mammals (*M*), and cocoons emptied by something other than predation by small mammals (*H*) observed in soil samples collected from October 2009 to 2012 at eight larch plantations in the University of Tokyo Hokkaido Forest

Category	Generation	Site								Average
		1	2	3	4	5	6	7	8	
<i>I</i>	2009	6.6 (3-15)	5.0 (1-10)	13.6 (0-60)	8.0 (0-28)	4.6 (0-20)	47.4 (17-73)	24.1 (11-51)	8.2 (2-27)	14.7
	2010	37.9 (12-107)	35.8 (4-81)	21.5 (5-50)	8.9 (1-18)	1.5 (0-7)	8.3 (3-18)	6.4 (3-18)	29.3 (9-77)	18.7
	2011	14.9 (3-34)	10.0 (3-18)	6.0 (0-19)	1.3 (0-6)	0.3 (0-2)	21.1 (0-66)	20.3 (4-66)	15.0 (6-35)	11.1
	2012	0.4 (0-3)	7.4 (1-23)	3.7 (0-24)	0.0 (0)	0.1 (0-1)	12.6 (4-24)	16.8 (3-56)	2.6 (0-11)	5.45
<i>M</i>	2009	0.0 (0)	0.7 (0-3)	0.1 (0-1)	0.0 (0)	0.0 (0)	9.0 (0-25)	3.8 (0-14)	0.0 (0)	1.70
	2010	8.7 (2-18)	6.9 (0-27)	3.9 (0-12)	0.9 (0-5)	1.7 (0-8)	37.6 (17-72)	16.5 (3-37)	3.6 (1-11)	9.98
	2011	37.6 (11-94)	24.4 (2-79)	10.4 (0-21)	1.6 (0-6)	3.6 (0-9)	62.5 (8-102)	18.0 (0-34)	11.8 (2-45)	21.2
	2012	34.5 (2-58)	22.8 (4-77)	14.5 (0-32)	3.8 (0-10)	5.2 (0-24)	60.7 (31-125)	18.1 (5-31)	16.3 (6-29)	22.0
<i>H</i>	2009	0.2 (0-1)	0.5 (0-2)	0.4 (0-2)	0.4 (0-1)	0.2 (0-1)	4.5 (2-8)	6.3 (2-16)	0.0 (0)	1.56
	2010	8.6 (4-17)	16.1 (6-27)	20.5 (4-40)	7.5 (0-27)	2.6 (0-10)	25.5 (10-52)	17.9 (3-34)	16.8 (6-30)	14.4
	2011	33.6 (11-73)	24.0 (6-50)	22.5 (7-46)	4.5 (0-14)	2.0 (0-5)	23.8 (6-67)	22.1 (0-57)	16.3 (4-34)	18.6
	2012	29.5 (3-52)	31.3 (9-62)	15.1 (0-55)	2.3 (0-7)	5.5 (0-21)	46.8 (16-60)	24.3 (8-75)	42.6 (27-67)	24.7
mean (minimum-maximum) (/0.04 m <sup>2</sup> )										



**Table 2-4** The number of newly spun cocoons ( $N$ ) per 0.04 m<sup>2</sup>, predation rates by small mammals before and after October sampling ( $\theta$  and  $\kappa$ ), and the annual remaining rate of empty cocoons ( $\phi$ ) estimated by a Bayesian hierarchical model from 2009-2012 at eight larch plantations in the University of Tokyo Hokkaido Forest. The predation rate throughout the cocoon period ( $\rho$ ) was estimated from posterior distribution of  $\theta$  and  $\kappa$

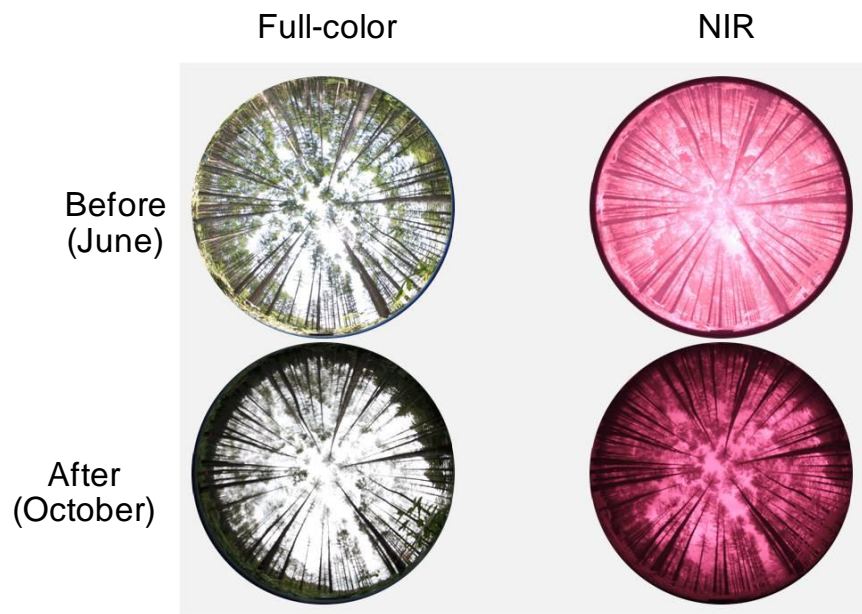
Parameter	Generation	Site							
		1	2	3	4	5	6	7	8
$N$	2009	12.67 (7.25-19.60)	10.73 (5.98-16.90)	9.95 (5.18-16.39)	10.78 (5.88-17.17)	8.60 (4.45-14.27)	59.10 (43.58-76.43)	31.92 (21.00-44.42)	13.22 (7.80-20.17)
	2010	46.97 (33.79-61.82)	39.39 (27.27-53.74)	27.17 (17.80-38.88)	14.01 (8.27-21.40)	4.41 (1.85-8.23)	25.88 (13.99-40.69)	17.15 (9.10-28.10)	33.32 (22.18-45.78)
	2011	32.59 (17.69-51.21)	19.68 (11.06-32.06)	10.81 (5.67-17.83)	4.34 (1.83-8.04)	3.57 (1.32-7.53)	38.88 (23.82-56.22)	23.36 (14.62-35.01)	21.90 (13.25-31.92)
	2012	6.23 (1.91-15.57)	14.11 (7.87-23.34)	6.55 (2.98-11.82)	3.62 (1.39-7.59)	3.82 (1.47-7.64)	27.90 (14.43-45.74)	21.15 (12.88-31.40)	13.29 (5.84-23.23)
$\theta$	2009	0.032 (0.010-0.081)	0.048 (0.009-0.151)	0.029 (0.008-0.076)	0.032 (0.009-0.083)	0.044 (0.011-0.114)	0.149 (0.085-0.225)	0.095 (0.037-0.169)	0.028 (0.008-0.074)
	2010	0.185 (0.108-0.269)	0.132 (0.064-0.212)	0.101 (0.023-0.197)	0.051 (0.011-0.131)	0.194 (0.024-0.476)	0.540 (0.239-0.725)	0.402 (0.080-0.650)	0.104 (0.031-0.189)
	2011	0.437 (0.115-0.659)	0.256 (0.036-0.536)	0.191 (0.018-0.464)	0.184 (0.024-0.474)	0.716 (0.262-0.938)	0.514 (0.314-0.663)	0.127 (0.022-0.303)	0.082 (0.016-0.224)
	2012	0.804 (0.276-0.971)	0.266 (0.039-0.534)	0.275 (0.030-0.620)	0.866 (0.417-0.986)	0.844 (0.357-0.977)	0.398 (0.078-0.650)	0.145 (0.020-0.339)	0.705 (0.433-0.857)
$\kappa$	2009	0.106 (0.025-0.281)	0.054 (0.017-0.121)	0.092 (0.025-0.215)	0.075 (0.016-0.193)	0.368 (0.120-0.644)	0.454 (0.256-0.629)	0.373 (0.116-0.604)	0.081 (0.020-0.205)
	2010	0.343 (0.078-0.590)	0.528 (0.274-0.743)	0.395 (0.144-0.655)	0.240 (0.047-0.525)	0.584 (0.109-0.955)	0.520 (0.059-0.928)	0.534 (0.102-0.941)	0.567 (0.272-0.847)
	2011	0.484 (0.083-0.902)	0.224 (0.035-0.596)	0.838 (0.344-0.980)	0.679 (0.139-0.966)	0.287 (0.021-0.817)	0.174 (0.030-0.461)	0.245 (0.040-0.583)	0.081 (0.016-0.233)
	2012	NA	NA	NA	NA	NA	NA	NA	NA
$\rho$	2009	0.135 (0.046-0.306)	0.100 (0.040-0.209)	0.118 (0.047-0.243)	0.105 (0.035-0.224)	0.396 (0.151-0.655)	0.535 (0.365-0.690)	0.433 (0.190-0.644)	0.107 (0.038-0.226)
	2010	0.465 (0.249-0.667)	0.590 (0.370-0.778)	0.456 (0.228-0.689)	0.279 (0.079-0.553)	0.665 (0.245-0.966)	0.782 (0.507-0.968)	0.724 (0.350-0.965)	0.612 (0.350-0.862)
	2011	0.709 (0.373-0.949)	0.424 (0.138-0.740)	0.868 (0.450-0.985)	0.737 (0.275-0.973)	0.798 (0.393-0.972)	0.599 (0.422-0.763)	0.341 (0.111-0.652)	0.157 (0.044-0.342)
	2012	NA	NA	NA	NA	NA	NA	NA	NA
An annual remaining rate of empty cocoons ( $\phi$ ) = 0.743									
Posterior mean (95% CIs)									
NA: not available									

**Table 2-5** Estimated numbers of adults and females of *Pristiphora erichsonii* (Hartig) at eight larch plantations in the University of Tokyo Hokkaido Forest (2009-2012)

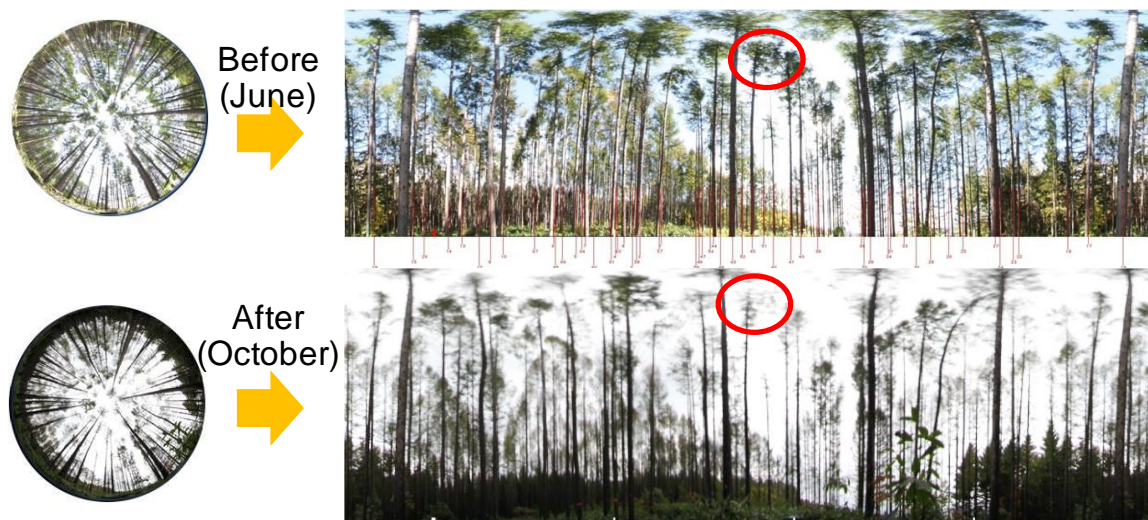
Generation	Density (/m <sup>2</sup> )								% Females
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	
2009	167.0 (163.7)	113.4 (111.1)	94.2 (92.3)	111.4 (109.1)	70.6 (69.2)	431.6 (423.0)	154.2 (151.1)	116.1 (114.3)	98
2010	412.5 (383.6)	204.6 (190.3)	190.9 (177.5)	94.0 (87.4)	19.7 (18.3)	77.8 (72.3)	31.3 (29.1)	80.5 (74.9)	93
2011	60.4 (5.8)	36.9 (35.4)	10.0 (9.6)	6.6 (6.3)	0	194.2 (186.4)	142.2 (136.5)	175.5 (168.5)	96
*2012	2.5 (2.5)	2.5 (2.5)	0	0	0	25.0 (25.0)	5.0 (5.0)	2.5 (2.5)	100

\*: small mammal predation after sampling was not considered in the value of generation 2012

Adults (females)



**Figure 2-1** Full-color and the near-infrared (NIR) photos that were taken in June (before insect defoliation) and in October (after insect defoliation)



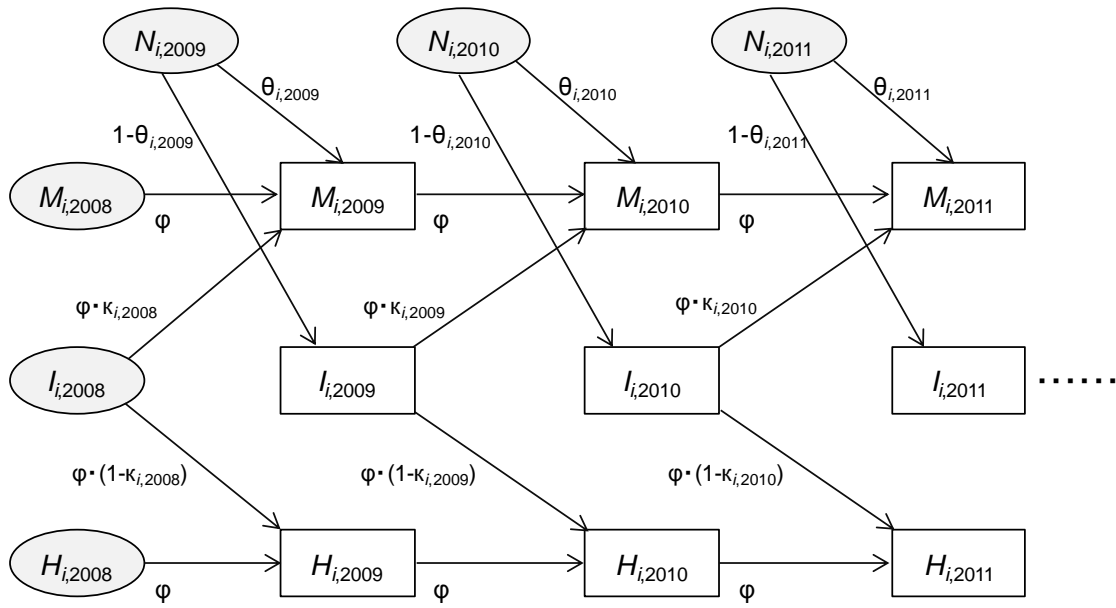
**Figure 2-2** Full color and the near-infrared (NIR) panorama photos that were transformed from canopy photos and used for the evaluation of defoliation



**Figure 2-3** Head capsules of instar I-IV of *Pristiphora erichsonii* (Hartig). HW: width of head capsule

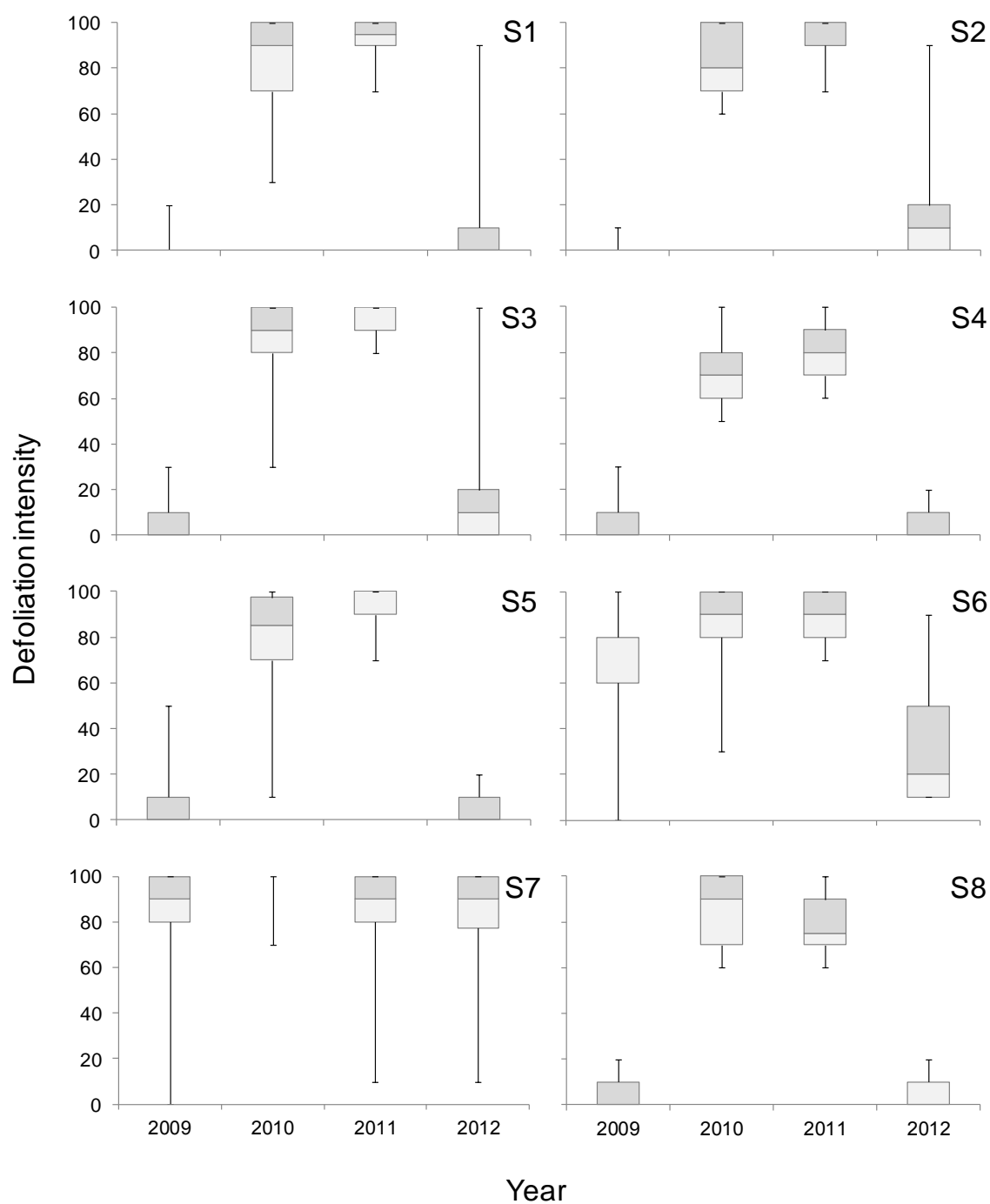


**Figure 2-4** *Pristiphora erichsonii* (Hartig) cocoons showing different appearance of the hole caused by the following: **A**, Normal emergence of *P. erichsonii* adult; **B**, parasitic wasp; **C**, parasitic fly; and **D**, predation by small mammals



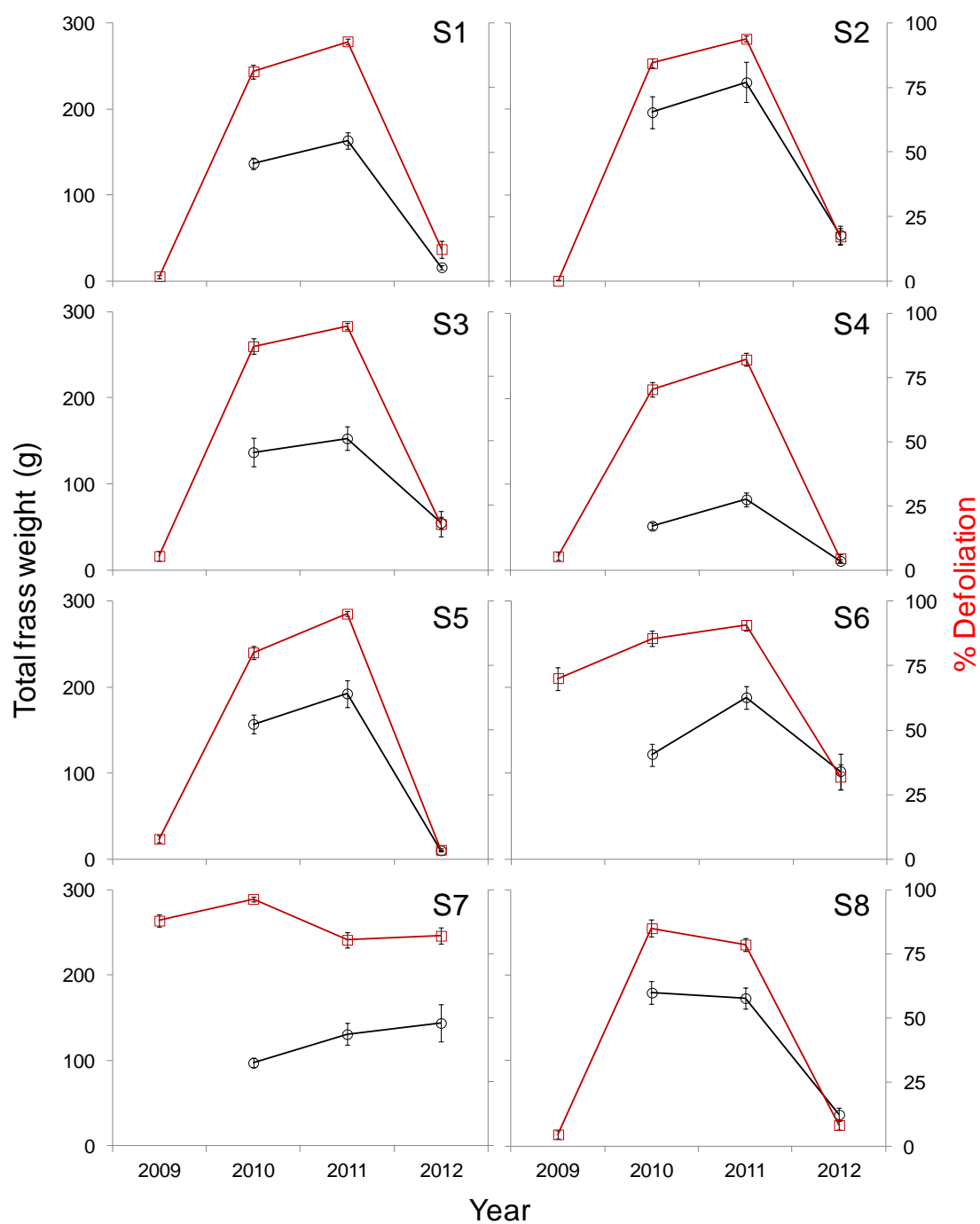
**Figure 2-5** Cocoon dynamic model.

$N_{i,t}$ , newly spun cocoons in the summer of generation  $t$ ;  $\theta_{i,t}$ , predation rate by small mammals before October sampling of generation  $t$ ;  $\kappa_{i,t}$ , predation rate by small mammals after October sampling of generation  $t$ ;  $\phi$ , annual remaining rate of empty cocoons;  $M_{i,t}$ , empty cocoons due to small mammal predation in October samples of year  $t$  including previous generations;  $I_{i,t}$ , unopened cocoons spun in year  $t$  and found in October samples of the same year, which include healthy-looking cocoons and the current generation's cocoons with mycelia;  $H_{i,t}$ , cocoons emptied by something other than small mammal predation in October samples of year  $t$ , which consisted of previous generations

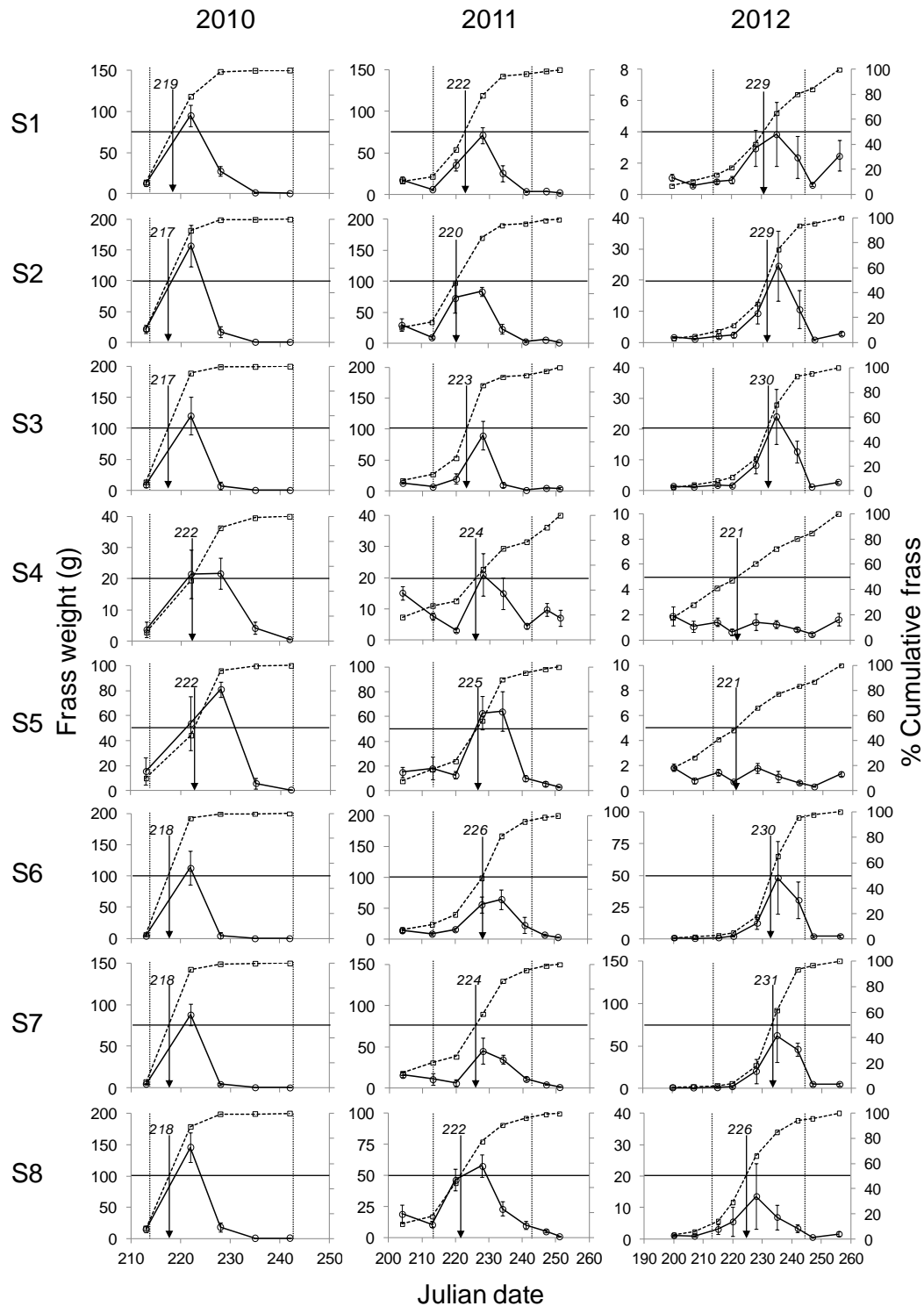


**Figure 2-6** Box and whisker plot diagram displaying defoliation intensity of larch tree at eight research sites in the University of Tokyo Hokkaido Forest, Japan (2009-2012)

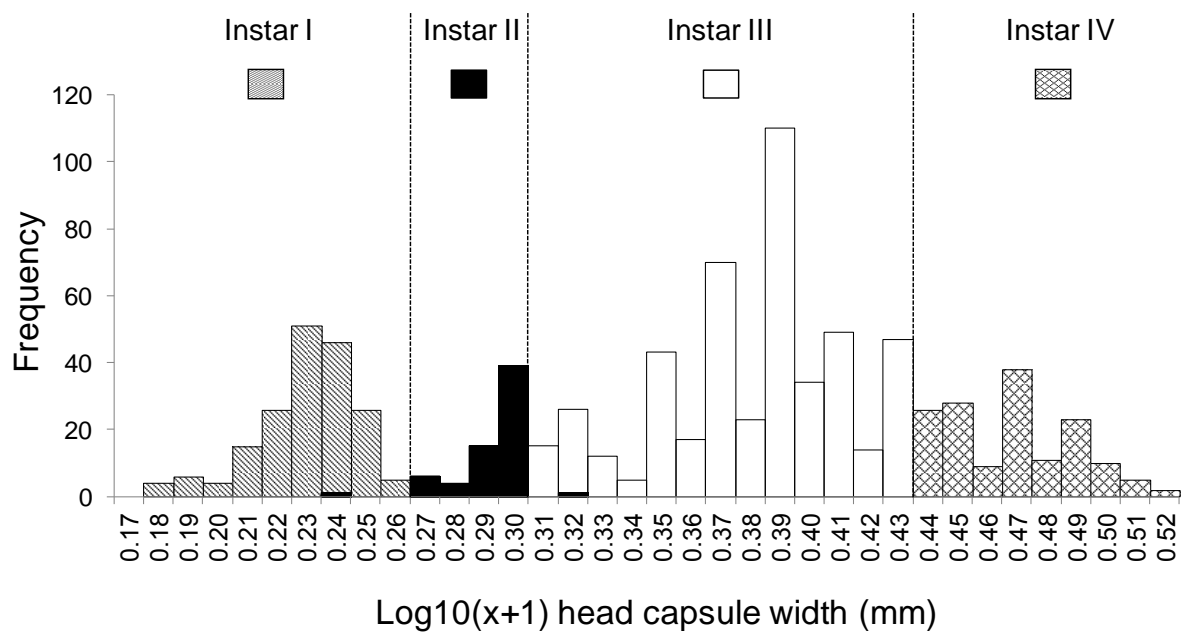




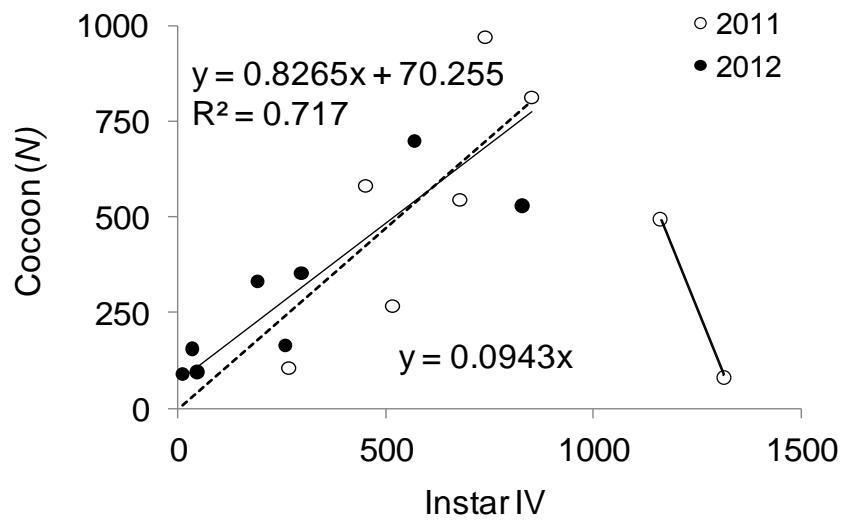
**Figure 2-7** Annual changes in total weight of frass production by *Pristiphora erichsonii* (Hartig) (a black line with open circles) and in defoliation intensity (a red line with open squares) at eight research sites in the University of Tokyo Hokkaido Forest, Japan (mean  $\pm$  SE)



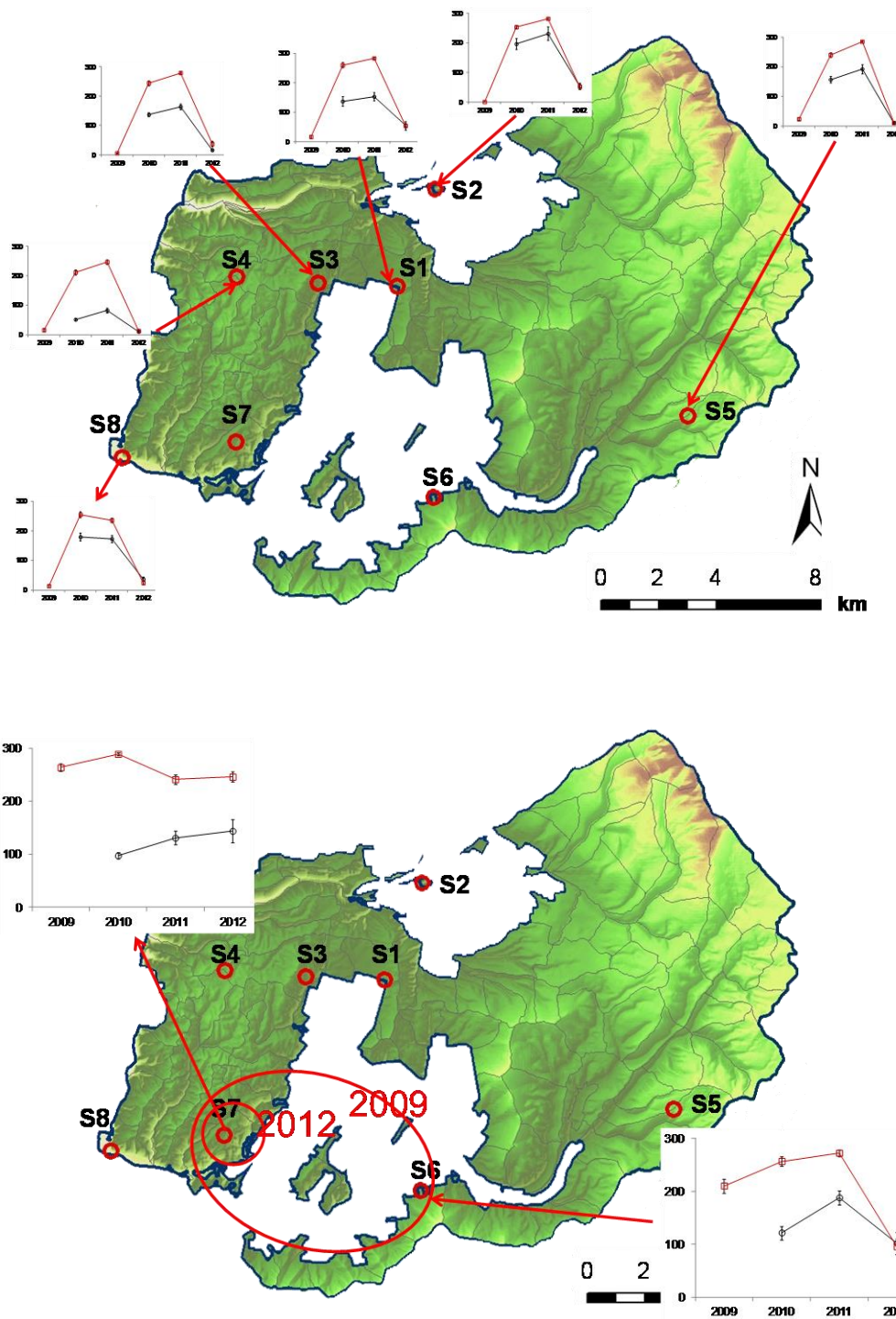
**Figure 2-8** Seasonal incidence and frass yield caused by *Pristiphora erichsonii* (Hartig) defoliation at eight research sites. Solid line with circle marks: the mean frass weight per litter trap ( $m^2$ )( $\pm$  SD). Dashed line with square marks: the mean cumulative frass weight (percentage). Arrow point down: Julian date at the 50% cumulative falling frass. Dotted line: the beginning of each month



**Figure 2-9** Histogram of larval head capsule widths of *Pristiphora erichsonii* (Hartig) for each instar identified by color



**Figure 2-10** Relationship between densities of instar IV and of newly spun cocoon ( $N$ ) at eight larch plantations in the University of Tokyo Hokkaido Forest from 2011 to 2012. Because no significant correlation was found for all data ( $r=0.433114$ ,  $P>0.05$ ), two significant regression lines were obtained separately depending on density of instar IV; one was negative correlation for two points with great density of instar IV, the other was positive correlation for 14 points other than the two



**Figure 2-11** Spatial and temporal population dynamics of *Pristiphora erichsonii* (Hartig) at eight research sites in the University of Tokyo Hokkaido Forest, Japan, during 2009-2012. A black line with open circles indicates frass production by *P. erichsonii* and a red line with open squares indicates defoliation intensity

## CHAPTER 3: NATURAL ENEMIES

### 3.1. Introduction

Morris (1957) observed that the importance of mortality as a determinant of insect population abundance was determined not by the amount of mortality but by the mortality variations that are related to changes in insect population densities. Direct density dependence can be an important cause of temporal density dependence if it begins to exert a regulatory influence on host population densities (Hassell 1998; Murdoch 1994; Walde and Murdoch 1988). Such a form of population regulation, a combination of small-scale manipulation and large-scale observation over time provides both the mechanism causing density dependent mortality and how these processes are likely to cope with population regulation.

Since *P. erichsonii* spends most of the year inside a cocoon in soil (Ives and Turnock 1959; Lejeune 1955), many researchers paid attention to the important mortality factors affecting the cocoon stage. However, it is very likely that rates of small mammal predation were overestimated such in case of Tachibana and Nishiguchi (1984).

In the previous chapter, four successive years (2009-2012) of cocoon sampling from the soil were conducted in October, when all individuals of *P. erichsonii* were supposed to have spun cocoons. The cocoons were separated into three categories: unopened cocoons, empty cocoons caused by small-mammal predation, and empty cocoons caused by something other than small-mammal predation. In this chapter, the unopened cocoons were incubated to record emergence of living inhabitant under laboratory condition. Each of the mortality factors was determined how they operated with *P. erichsonii* density. Parasitic wasps, parasitic flies, and entomopathogenic fungi were examined as parasites on *P. erichsonii* cocoons. Relationships among *P. erichsonii* cocoon abundance, abundance of small mammals, and their predation on the cocoons were assessed.

Regarding predators on free-living larval stage, predators attacking *P. erichsonii* larvae that had been artificially attached to the branch were determined.

### 3.2. Materials and Methods

#### 3.2.1. Small mammal data

Small mammal trapping data was obtained from the regular monitoring of forest pests by the UTHF, which was authorized by the Kamikawa Branch Office, Hokkaido Prefectural Government under the Wildlife Protection and Proper Hunting Act (<http://law.e-gov.go.jp/htmldata/H14/H14HO088.html>; Ministry of the Environment, Japan). Small mammal trapping was conducted in early June (spring) and mid-September (fall) of every year since 2000. The data from June 2000 to June 2013 were used in this study. Fifty snap traps (PANCHU®, Otsuka, Osaka, Japan) containing peanuts as bait were deployed in two 5 by 5 grids at LP and LN sites and in 5 by 10 grid at HP and HN sites. The grid spacing in both cases was 10 m. The traps were set for three nights for each survey. The traps were checked daily in the morning. Captured mammals were transferred to the laboratory and were identified into the taxa level. Predominant taxa included the small Japanese field mouse (*Apodemus argenteus*), the large Japanese field mouse (*Apodemus speciosus*), the grey red-backed vole (*Myodes rufocanus bedfordiae*), and shrews (*Sorex* spp.). The PANCHU® is a type of snap trap that kills a small mammal within three minutes and conforms to the “Guidelines of the American Society of Mammalogists for the use of wild mammals in research” (Sikes and Gannon 2011). No red data species have been collected since 2010. The total numbers of captures per 100 traps per three nights were used for in the analyses.

### **3.2.2. Assessment of biotic mortality factors during cocoon stage**

*P. erichsonii* cocoons that were sorted from soil samples and incubated in planter during winter and in plastic tube after overwintering in Chapter 2 were used in this study. An emergence of living inhabitant was checked twice a week. Each emerged inhabitant, including *P. erichsonii* adult, parasitic fly, and parasitic wasp, was recorded. Diseased cocoons were individually incubated for 2-7 days at 25 °C, followed by isolation onto a potato dextrose agar (PDA) plate. The isolated fungus growing on the PDA plate was identified microscopically or using molecular technique. The cocoon that did not yield *P. erichsonii* adult or a parasite until the end of August was dissected. If a larva inside a cocoon was found alive, it was judged as those in prolonged diapauses. Mortality caused by small mammal predation was determined by a hierarchical Bayesian model (cf. subsection 2.2.4).

### 3.2.3. Predators during larval stage

In August of 2011, *P. erichsonii* larvae (approximately 1,000 larvae) were shifted on a single larch branch of a larch tree at site 5 then covered with nylon mesh bag to prevent from predation. On the following day a video recording camera was fixed after removing the mesh bag. The video recording of predation event was conducted from 9.00 AM to 19.00 PM (Fig. 3-1A). A litter trap with an opening 1m<sup>2</sup> (1m x 1 m) was placed beneath the branch to collect remains of cadavers, in which the predators do not consume all of its prey (Fig. 3-1B). A distinctive mark on cadaver was used to identify the perpetrator. For example, wasps leave a head of *P. erichsonii* larvae (Muldrew 1955), a pair of small holes results from Neuroptera, a single hole from Heteroptera, and a peppering of small holes from ants (Mills 2007).

### 3.2.4. Statistical analysis

The relationships between the trap captures of small mammals and the three predation rates ( $\theta$ ,  $\kappa$ , and  $\rho$ ) were examined using a beta regression, with the trap captures as being as an explanatory variable and predation rates as a response variable. The fall trap captures were used for the predation rate before the October sampling ( $\theta$ ). The spring trap captures were used for the predation rate after the October sampling ( $\kappa$ ). The average of fall captures in a given year and spring captures of the following year was used to calculate the total predation rate ( $\rho$ ). The three relationships were obtained by using the trap captures of the four species (*A. argenteus*, *A. speciosus*, *M. r. bedfordiae*, *Sorex* spp.) and their total. Coefficient and its *P*-value were determined.

The Pearson product-moment correlations were obtained for all combinations of trap captures of each taxon of small mammals. The correlation was separately determined for fall, spring, and an average of fall and spring.

I also assessed whether cocoon abundance, site, and year had effects on the three predation rates using a beta regression. In the generalized linear model, with a beta error structure and a logit link function (Stroup 2013), cocoon abundance, year, and site being use an explanatory variables of a full model and predation rates as a response variable. A null model contained only random effects. The best model was determined by comparing Akaike's Information Criterion (AIC) from the full model to the null model by



changing explanatory variables. A ‘betareg’ library (Zeileis et al. 2013) in R ver. 2.15.3 was used for the beta regression.

Effects of cocoon abundance, site, and year on a rate of survival or a rate of each of common identified mortality factors obtained by incubation were also analyzed by beta regression. Two datasets were used for the analysis: One included small mammal predation before sampling ( $\theta$ ) because the incubated cocoons had been exposed to natural enemies after spinning cocoons until sampling. The other included small mammal predation through the cocoon period ( $\rho$ ) to evaluate mortality of whole period of cocoon stage. The percentage of diseases was underestimated whereas the percentage of parasitoids did not increase after spinning cocoons because they parasitize during a stage of free-living larva.

### 3.3. Results

#### 3.3.1. Small mammal data

Trap captures of each taxon of small mammals increased in the summer (spring trapping to fall trapping) but decreased in the winter before 2009 (Fig. 3-2). However, the abundance of small mammals began to increase after 2009. The increase was small for *Sorex* spp. but greater for *A. speciosus*, *A. argenteus*, and *M. r. bedfordiae*. Trap captures decreased greatly in spring 2012 for *A. speciosus* and in spring 2013 for *A. argenteus*. Regarding *M. r. bedfordiae*, trap captures shown a small decrease in spring 2012 and a large decrease in spring 2013.

Rates of predation by small mammals tended to increase with trap captures, with the exception of *Sorex* spp. (Table 3-1): coefficients were positive for *A. argenteus*, *A. speciosus*, *M. r. bedfordiae* and total of the three species, but negative for *Sorex* spp. These coefficients are significantly differed from 0 with three exceptions out of 15 ( $P < 0.05$ ).

The Pearson product-moment correlation coefficient of trap captures was significant for 12 of the 18 combinations between different small mammal taxon ( $P < 0.05$ ). Of these, negative correlations were found in *A. argenteus*-*Sorex* spp., *M. r. bedfordiae*-*Sorex* spp. for fall, *A. speciosus*-*Sorex* spp. for spring, *A. speciosus*-*Sorex* spp. and *M. r. bedfordiae*-*Sorex* spp. for the average of fall and spring (Table 3-2).

A model with “year” was selected as the best model to explain the rate of small-mammal predation before October sampling ( $\theta$ ), after October sampling ( $\kappa$ ), and a predation rate throughout a

season ( $\rho$ ) (Table 3-3). Among models that include cocoon abundance as an explanatory variable, a model with a main effect of cocoon and year was best for all the three predation rates. The model with a main effect of cocoon and year was second best for  $\kappa$  ( $\Delta AIC=2.12$ ) and for  $\rho$  ( $\Delta AIC=7.06$ ). However, for  $\theta$ , the second best model had a main effect of year and site ( $\Delta AIC=12.5$ ), and a model with a main effect of cocoon and year was third ( $\Delta AIC=12.2$ ).

### 3.3.2. Assessment of biotic mortality factors during cocoon stage

Six species of parasitoids emerged from *P. erichsonii* cocoons that were collected from forest soils in October and incubated in planters for overwintering and in laboratory thereafter. Of these, three species were parasitic wasps and the other three species were parasitic flies, including *Aptesis* sp., Tribe Mesoleiini (resembled *Lamachus* sp. but need more identify), *Endasys* sp., *Myxexoristops stolidus* (Stein), *Vibrissina turrita* (Meigen) and *Bessa parallela* (Meigen) (Fig. 3-3). Entomopathogenic fungi that were isolated from field-collected cocoons are as follows; *Beauveria bassiana* (Bals.) Vuill., *B. brongniartii* (Sacc.) and *Isaria farinosa* (Holm.) (Fig. 3-4).

Spatial density dependence of each of the common identified mortality factors including survival rate were examined by plotting the percentage of each of them against abundance of newly spun cocoons ( $N$ ) (Fig. 3-5). Mortality factors were attributed to small mammal predation before sampling (SMP:  $\theta$ ), entomopathogenic fungi (EF), parasitic wasps (PW), parasitic flies (PF), and unknown factors (UN). The percentage of the larch sawfly adults (LSF) tended to decrease with year. The percentage of small-mammal predation increased greatly with year indicating its great contribution to the decrease of LSF. The UN, which shown much greater percentage than the other three mortality factors, was small in 2011. The percentages of other three mortality factors were mostly  $< 6\%$ . The PW and the PF tended to decrease with year. Results of beta regression are shown in Table 3-4, the UN was influenced by “year+site”. However, the LSF was influenced by “year+cocoon”. The PF was influenced by “cocoon”. The PW was influenced by “year”.

By considering small-mammal predation throughout the cocoon stage (SMP:  $\rho$ ) the situation has changed (Fig. 3-6). No conspicuous or consistent patterns were found in EF, PW, PF, and UN. Mortality by the small mammal predation was greatest followed by UN. The other three mortality factors were small. The number of LSF had negative relationship with the cocoon abundance in 2009 but positive relationship

in 2011. Overall the three years, proportion of LSF tended to decrease with year. The percentage of small mammal predation tended to increase with year. On the contrary to the LSF, spatial density dependence of small mammal predation was positive in 2009 but negative in 2011. A model with “year” was selected as an effect of the best model to explain the mortality caused by PW and survival of LSF, whereas a model with an effect of “site” was best for EF. The model using year and site as explanatory variables was best for UN. “Cocoon abundance” was included in the best models for PF (Table 3-5).

### **3.3.3. Predators and parasitoids during larval stage**

There were no avian predators attacking on *P. erichsonii* larvae. Dragonfly was only the one predatory insect appeared in the video but did not attack on the larvae. Furthermore, no distinctive mark on the body of falling larvae or cadavers was found in this observation.

## **3.4. Discussion**

Cocoon is one of the most vulnerable stages in sawfly life cycle. Some species of sawflies overwinter in the cocoon stage whereas the others spend an extended period of time during summer in this stage (Dahlsten 1967). Overwintering *P. erichsonii* cocoons are exposed to a number of predators, parasitoids, and entomopathogenic fungi. Especially, small-mammal predation on sawfly cocoons have been observed for a long period (Graham 1928; Hanski and Parviainen 1985; Holling 1959). In addition, Chapter 2 in this study shown that the decreasing between instar IV and cocoon indicates mortality in instar V. Great mortality was found during instar V in some cases but usually occurred during cocoon period.

The dataset covers a 13-year period (2000-2013) of 2-season surveys for small mammals and a 3-year period (2009-2012) of yearly surveys for *P. erichsonii* cocoons. By 2009, the abundance of small mammals increased in the summer (spring trapping to fall trapping) but decreased in the winter, which suggests that small mammals decreased in winter probably due to a food shortage. However, the trend was changed in 2010-2012. After the current population outbreak of *P. erichsonii* began, abundance of small mammals did not always decrease in spring captures. *A. argenteus* (Fig. 3-2A) and *M. r. bedfordiae* (Fig. 3-2B) tended to increase continuously, likely due to a high number of *P. erichsonii* cocoons that possibly had acted as a supplemental diet for these small mammals in winter, improving their survivorship. A

similar increase in population was observed for *A. speciosus* and *Sorex* spp. However, the response of *Sorex* spp. was small. Results on *A. argenteus*, *A. speciosus* and *M. r. bedfordiae* suggested a numerical response to *P. erichsonii* cocoons. A numerical response of small mammals has been reported for *A. speciosus* to *P. erichsonii* in central Japan (Tachibana et al. 1984) and for *Sorex* and *Peromyscus* to the European pine sawfly, *Neodiprion sertifer* in Canadian pine plantations (Holling 1959). Hanski (1988) considered that a numerical response in small mammals to *N. sertifer* was unlikely because *N. sertifer* cocoons are accessible to small mammals for only a short period of time in late summer. Hanski (1990) mentioned that “Holling (1959) worked in pine plantations with little alternative food for insectivorous and omnivorous small mammals and during *N. sertifer* outbreak, when large numbers of cocoons probably stayed in prolonged diapause during most of the year (Hanski 1988) and allowed an increase in small mammal numbers.” This situation seemed similar to my case of *P. erichsonii*: cocoons of *P. erichsonii* generally stayed approximately 10 months in soil without prolonged diapause though a small percentage of the cocoons (< 1%; Panisara Pinkantayong, personal observation) underwent prolonged diapause to stay in soil for two winters or more. In winter, the supply of alternative food for small mammals was poor as indicated by the winter decrease in small mammal abundance observed before 2009 (Fig. 3-2).

The trap captures of each of the four small mammal taxa fluctuated in a similar manner among the four sites (Fig. 3-2). It has been reported that the populations of these small mammal species widely show population synchrony (Bjørnstad et al. 1999; Stenseth et al. 1996). In Hokkaido, the scale of the regional level of population synchrony for *A. speciosus* was reported to be approximately 25 km (Bjørnstad et al. 1999). The distances between any two of the eight cocoon sampling sites and small mammal trapping sites were shorter than the range (Fig. 1-3), so the numbers of each small mammal species are likely to have fluctuated in a similar manner to the number of trap captures. Even more surprising is that the numbers of small mammals in the study area could be sustained by *P. erichsonii* cocoons that were distributed in small clumps in the landscape, depending on the distribution of larch plantation patches (Fig. 1-3). Larch plantations were smaller than 1% of the total forest area and were scattered throughout the UTHF (Fig. 1-3), though there were some small patches (< 1 ha each) of larch plantation outside of the UTHF territory. The home range of *Apodemus* spp. has been estimated to be approximately 60 m in radius or smaller (Lee et al. 2012; Vukićević-Radić et al. 2006), with seasonal variation and differences by sex. Results of my

present study suggest that two *Apodemus* species and *M. r. bedfordiae* are likely to move more widely than the reported home range.

Predation by small mammals tended to increase with the abundances of *A. argenteus*, *A. speciosus*, and *M. r. bedfordiae* whereas trap captures of *Sorex* spp. had significant negative effects on the predation rates (Table 3-1). Because the trap captures of *Sorex* spp. were negatively correlated with those of *Apodemus* spp. and *M. r. bedfordiae* (Table 3-2), significant negative effects of *Sorex* spp. on the predation rate were very likely to be an artifact that was caused by interaction of shrews with other ecologically similar mammals.

At a landscape level, population outbreaks of *P. erichsonii* promoted the population growth of small mammals (Fig. 3-2), which in turn resulted in a higher rate of cocoon predation by these mammals (Table 3-1). However, the best model to explain each of the small mammal predation rates ( $\theta$ ,  $\kappa$ , and  $\rho$ ) did not include *P. erichsonii* cocoon abundance but only “year” as an explanatory variable (Table 3-3). Difference in AIC suggested that the effects of the cocoon abundance on predation rate at each site was not so strong. These results also indicated that small mammals did not respond to cocoon abundance at a local scale but at larger scale such as landscape level, which was a likely cause that the best model included only “year” as an explanatory variable. These are consistent with a wide range of movement discussed above.

Insectivorous and omnivorous small mammals show strong preference for sawfly cocoons (Hanski 1990) because of their large size and high nutritional content (Barnard and Brown 1981; Dickman 1988; Hanski 1992; Platt and Blackley 1973). As a result, predation by small mammals against the prey density shows an S-shape curve (Holling’s type III functional response) (Holling 1959), in which the rate of predation first increased with increasing prey density, then decreased. According to beta regression results (Table 3-3), the model with a main effect of cocoon and year as explanatory variables was second best for  $\kappa$  and for  $\rho$  but third best for  $\theta$ . This difference was likely caused by a difference in accessible time: the accessible time was less than two months for the predation before October sampling ( $\theta$ ), while it was approximately 8 months for the predation after October sampling ( $\kappa$ ). In a case involving *N. sertifer*, for which cocoons were accessible to small mammals for a short period of time in late summer, the rate of predation by small mammals on cocoons was inversely density dependent on the cocoon abundance

(Hanski 1990). Similarly, it is likely that there was not enough time to respond to cocoon abundance for predation before October sampling ( $\theta$ ).

The increase in density of the three major small mammals (Fig. 3-2) was a likely cause of increased predation rates before October sampling, although the increase was most likely not enough to depress *P. erichsonii* population outbreaks, as reported in many articles (Buckner 1956; Drouin et al. 1968; Ives and Turnock 1959). Tachibana and Nishiguchi (1984) concluded that small-mammal predation was a major factor terminating population outbreaks of *P. erichsonii* in Nagano Prefecture, Japan because cocoon abundance began to decrease after the number of empty cocoons preyed on by small mammals became greater than the number of healthy-looking cocoons just before *P. erichsonii* adult emergence. However, results of this study did not support their conclusion for the following reasons: Firstly, Tachibana and Nishiguchi (1984) overestimated the number of cocoons emptied by small mammal predation because empty cocoons remains longer than one year as suggested as an annual remaining rate of empty cocoons ( $\phi = 0.743$ , Table 2-2) in this study. Secondly, in 2011, the rate of predation by small mammals at the six sites where cocoon abundance decreased significantly in 2012 was not larger than those at sites 6 and 7, where cocoon abundance did not decrease in 2012. In the six sites, insect defoliation was not severe in 2012 (Appendix 3, see also Table 4-1 in Chapter 4), indicating that great mortality occurred from the adult stage of the previous generation to the young larval stage in summer 2012.

Results on biotic mortality factors other than small mammals during cocoon stage were also analyzed herein. *P. erichsonii* cocoon mortality caused by parasites (parasitoids and entomopathogenic fungi) was small and lack of a consistent pattern (Fig. 3-5). Mortality factors other than small-mammal predation (except UN) were < 15.0% (the greatest PW was 13.0% at site 4 in 2010). Similarly, many studies have reported that the mortality by parasites was generally small with variation among species and locations (Dahlsten 1967). The percentage of parasitized cocoons ranged 1.75%-4% for hymenopterous parasites, 14.9%-25.4% for dipterous parasites (Richmond et al. 1995), 2.8%-23.5% for entomopathogenic fungi (MacLeod and Heimpel 1955), and 1.76% to 12.56% for total parasitism (Harman 1971; Ives 1976). Harman (1971) and Muldrew (1956) remarked poor synchronization of emerged parasite adults with life cycle of *P. erichsonii* was a likely cause of low parasitism, in which most of parasites emerged after most *P. erichsonii* larvae had entered to the soil and spun cocoons. In my study, adult parasitoids started to

emerge from early June though *P. erichsonii* adults did one month later suggesting that these parasitoids likely need alternative hosts to parasitize *P. erichsonii* larvae. This seems a likely cause why these parasitoids did not show numerical response or density dependence to *P. erichsonii* populations in my study with one exception of PF (Table 3-4) though the mortality was small to depress outbreak population of *P. erichsonii* (Fig. 3-5). The poor synchronization in life cycles between parasitoids and *P. erichsonii* may have depended on their origin. Originally, larches are not native of Hokkaido and many species of larches were introduced from the mainland of Japan (Honshu), Korea, Sakhalin, and Krill Islands. Higashiura (1988) assumed that *P. erichsonii* in Hokkaido had introduced with *L. kaempferi* (= *L. leptolepis*) from the Honshu because *P. erichsonii* in Hokkaido deposited smaller numbers of eggs on *L. gmelinii* var. *japonica* (= *L. dahurica*) and their hybrid (*L. gmelinii* var. *japonica* x *L. kaempferi*) than on *L. kaempferi*. Higashiura (1988) also considered the smaller numbers of oviposition by *P. erichsonii* on *L. gmelinii* var. *japonica* and the hybrid was due to asynchrony of *P. erichsonii* with these larches because timing of starting elongation of long shoots was earlier in these larches than in *L. kaempferi* and because long shoots of these larches had started lignification process when *P. erichsonii* started oviposition. The parasitoids that were found in my study may also have different origins from *P. erichsonii* though further studies using genetic approaches are needed.

**Table 3-1** Effects of trap captures of each taxon of small mammals on the predation rate ( $\theta$  = before the October sampling,  $\kappa$  = after the October sampling, and  $\rho$  = the predation rate throughout the cocoon period) obtained from a beta regression

Trap capture season	Response variable (Predation rate)	Explanatory variable (Small mammal taxon)	Coefficient	<i>P</i>
Fall	$\theta$	<i>Apodemus argenteus</i>	0.058	0.000
	$\theta$	<i>Apodemus speciosus</i>	0.011	0.438
	$\theta$	<i>Myodes rufocanus bedfordiae</i>	0.075	0.000
	$\theta$	<i>Sorex</i> spp.	-0.153	0.001
	$\theta$	All	0.020	0.002
Spring	$\kappa$	<i>Apodemus argenteus</i>	0.014	0.176
	$\kappa$	<i>Apodemus speciosus</i>	0.089	0.033
	$\kappa$	<i>Myodes rufocanus bedfordiae</i>	0.071	0.003
	$\kappa$	<i>Sorex</i> spp.	0.154	0.543
	$\kappa$	all	0.018	0.020
(Fall+Spring)/2	$\rho$	<i>Apodemus argenteus</i>	0.034	0.020
	$\rho$	<i>Apodemus speciosus</i>	0.077	0.010
	$\rho$	<i>Myodes rufocanus bedfordiae</i>	0.138	0.000
	$\rho$	<i>Sorex</i> spp.	-0.320	0.000
	$\rho$	all	0.021	0.014

$\theta$ : predation rate before October sampling

$\kappa$ : predation rate after October sampling

$\rho$ : predation rate throughout season



**Table 3-2** Pearson product-moment correlation coefficient for all combinations of trap captures of each taxon of small mammals

Season	Variable		<i>r</i>	<i>P</i>	<i>n</i>
Fall	Aa	As	0.31	0.084	32
	Aa	Mrb	0.99	0.000	32
	Aa	Sorex	-0.49	0.005	32
	As	Mrb	0.23	0.215	32
	As	Sorex	-0.17	0.353	32
	Mrb	Sorex	-0.44	0.013	32
Spring	Aa	As	-0.22	0.291	24
	Aa	Mrb	0.46	0.025	24
	Aa	Sorex	0.95	0.000	24
	As	Mrb	0.76	0.000	24
	As	Sorex	-0.52	0.009	24
	Mrb	Sorex	0.15	0.481	24
(Fall+Spring)/2	Aa	As	1.00	0.000	24
	Aa	Mrb	0.86	0.000	24
	Aa	Sorex	-0.37	0.076	24
	As	Mrb	0.90	0.000	24
	As	Sorex	-0.45	0.029	24
	Mrb	Sorex	-0.79	0.000	24

Aa: *Apodemus argenteus*, As: *Apodemus speciosus*

Mrb: *Myodes rufocanus bedfordiae*, Sorex: *Sorex* spp.

**Table 3-3** Akaike's Information Criteria (AIC) obtained by a beta regression to determine effects of cocoon abundance on the rate of small mammal predation at a local scale

Explanatory variable	Parameter					
	$\theta$		$\kappa$		$P$	
	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC
null model (predation rate ~1)	-13.7		-7.35		-0.38	
full model (cocoon + year + site)	-24.8	11.1	-0.01	-7.34	-4.30	3.92
cocoon + year	-25.9	12.2	-9.47	2.12	-7.44	7.06
cocoon + site	-3.94	-9.76	3.43	-10.8	8.96	-9.34
year + site	-26.2	12.5	-1.20	-6.16	-5.89	5.51
Cocoon	-12.2	-1.54	-6.07	-1.29	0.96	-1.34
Year	<b>-27.7</b>	14.0	<b>-11.5</b>	4.10	<b>-9.27</b>	8.89
Site	-3.60	-10.1	3.53	-10.9	7.87	-8.25

$\theta$ :  $n = 32$

$\kappa, \rho$ :  $n = 24$

Bold: best model

**Table 3-4** Akaike's Information Criteria (AIC) obtained by a beta regression to determine effects of cocoon abundance, year and site on the mortality factors when small mammal predation before sampling ( $\theta$ ) was included. Mortality factors such as entomopathogenic fungi (EF), parasitic wasp (PW), parasitic fly (PF), and unknown (UN) were shown. Effects on small mammal predation before sampling ( $\theta$ ) were shown in Table 3-3

Explanatory variable	Parameter									
	EF		PW		PF		UN		LSF	
	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC
null model (mortality~1)	-85.6		-166.2		-182.1		-26.5		-11.9	
full model (cocoon+year+site)	-81.5	-4.10	-165.3	-0.93	-182.4	0.33	-45.2	18.73	-20.3	8.34
cocoon+year	-83.7	-1.91	-172.3	6.10	-186.9	4.88	-25.2	-1.28	<b>-27.3</b>	15.4
cocoon+site	-83.5	-2.11	-157.5	-8.70	-182.5	0.49	-36.1	9.60	-7.40	-4.51
year+site	-83.4	-2.23	-166.9	0.71	-174.4	-7.65	<b>-45.4</b>	18.94	-21.0	9.11
Cocoon	-84.6	-1.04	-164.7	-1.48	<b>-189.8</b>	7.73	-25.8	-0.74	-14.2	2.29
year	<b>-85.6</b>	0.01	<b>-174.1</b>	7.92	-178.3	-3.73	-26.3	-0.18	-26.7	14.79
Site	-85.3	-0.33	-159.5	-6.71	-177.8	-4.24	-38.1	11.60	-8.60	-3.28

EF:  $n = 12$

PW, PF, UN, LSF:  $n = 24$

Bold: best model

**Table 3-5** Akaike's Information Criteria (AIC) obtained by a beta regression to determine effects of cocoon abundance, year and site on the mortality factors when small mammal predation throughout the cocoon period ( $\rho$ ) was included. Mortality factors such as entomopathogenic fungi (EF), parasitic wasp (PW), parasitic fly (PF), and unknown (UN) were shown. Effects on small mammal predation throughout the cocoon period ( $\rho$ ) were shown in Table 3-3

Explanatory variable	Parameter									
	EF		PW		PF		UN		LSF	
	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC	AIC	$\Delta$ AIC
null model (mortality~1)	-101.0		-177.6		-193.7		-29.5		-23.0	
full model (cocoon+year+site)	-98.4	-2.53	-177.0	-0.57	-190.3	-3.46	-36.4	6.87	-32.1	9.12
cocoon+year	-99.2	-1.75	-184.2	6.57	-197.1	3.32	-31.7	2.22	-36.4	13.4
cocoon+site	-100.4	-0.55	-167.9	-9.66	-191.3	-2.44	-27.8	-1.72	-17.6	-5.44
year+site	-100.4	-0.54	-178.1	0.54	-184.9	-8.82	<b>-38.2</b>	8.69	-34.1	11.1
cocoon	-100.5	-0.46	-176.0	-1.63	<b>-199.9</b>	6.17	-28.8	-0.72	-24.2	1.20
year	-100.9	-0.06	<b>-185.7</b>	8.08	-189.9	-3.80	-32.6	3.08	<b>-37.6</b>	14.6
site	<b>-102.4</b>	1.41	-169.9	-7.73	-188.4	-5.33	-27.9	-1.55	-19.6	-3.45

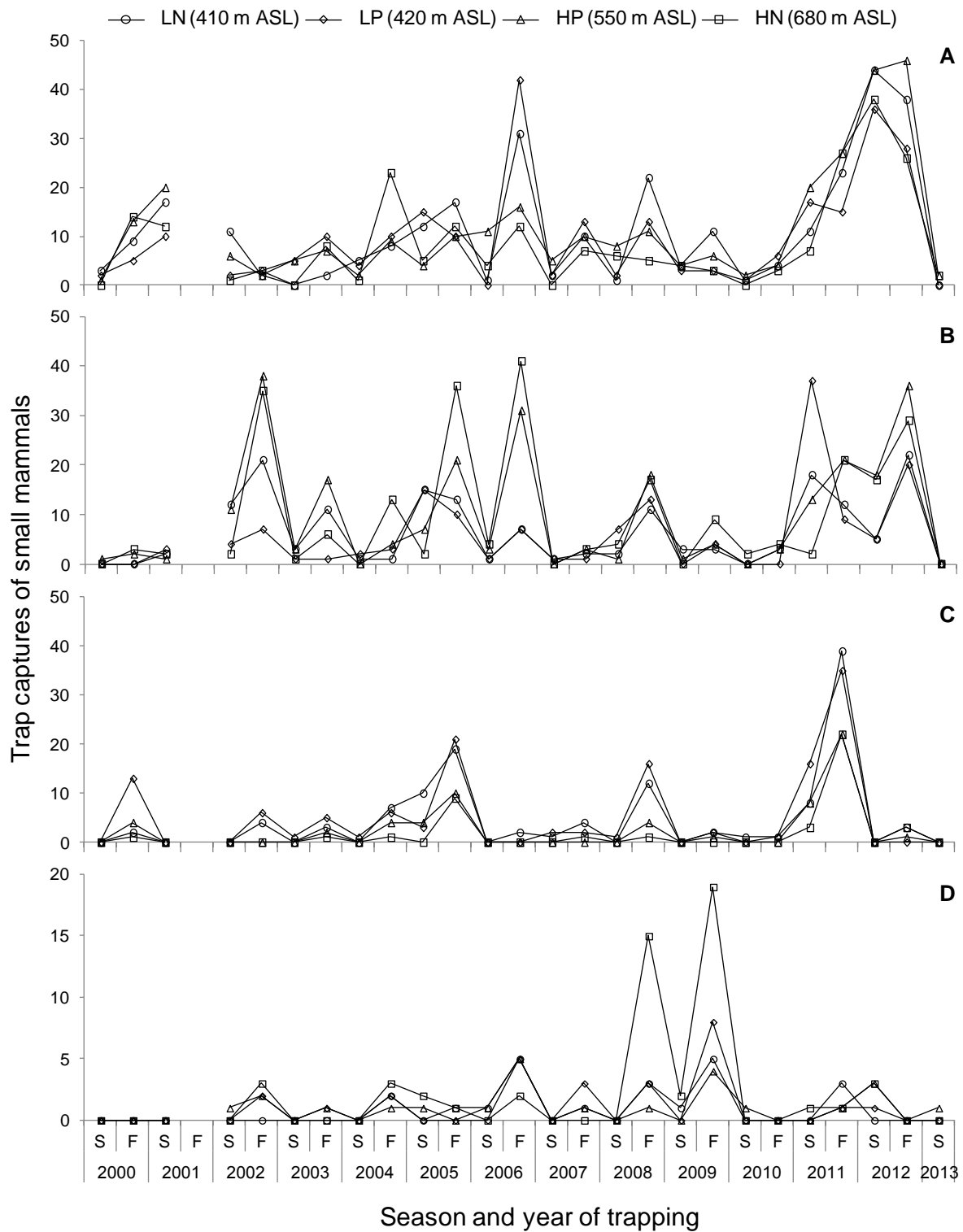
EF:  $n = 12$

PW, PF, UN, LSF:  $n = 24$

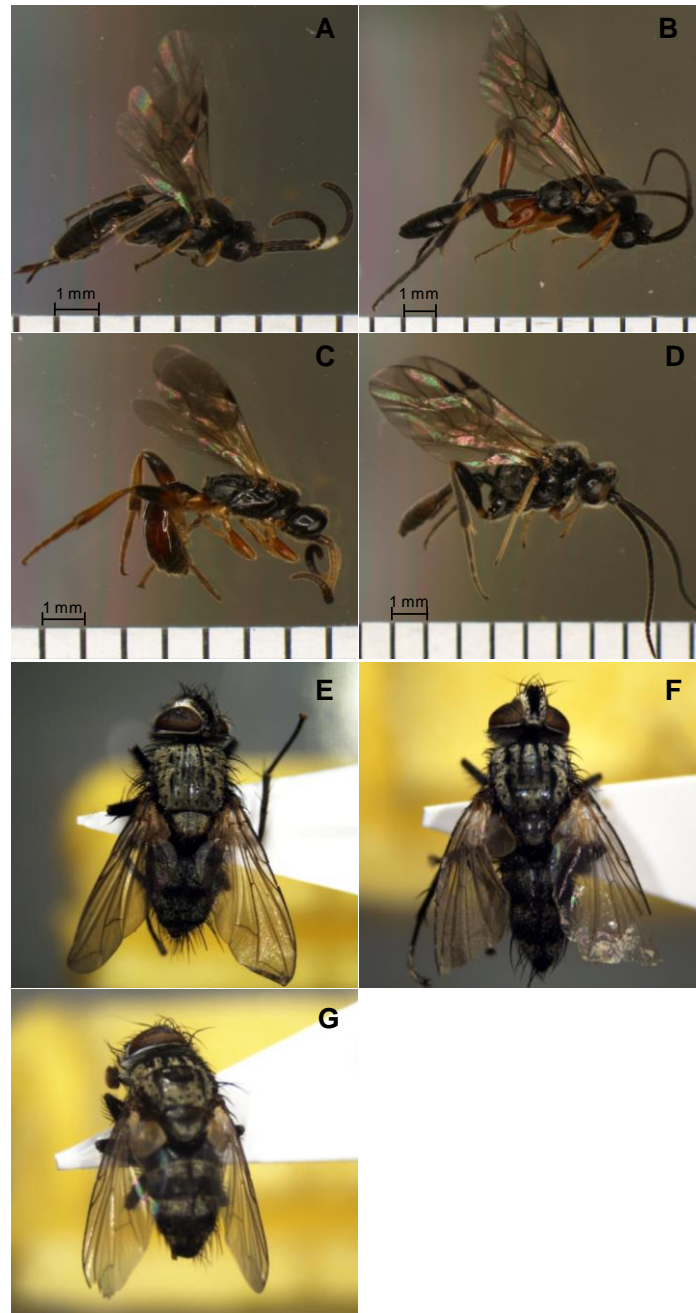
Bold: best model



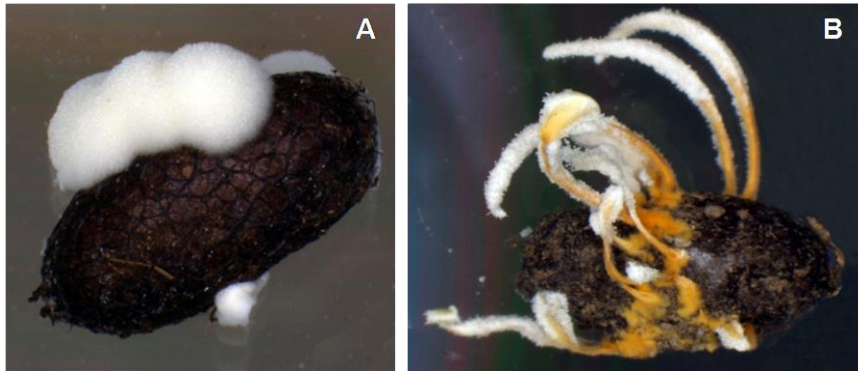
**Figure 3-1 A,** Monitoring the predator of *Pristiphora erichsonii* (Hartig) during larval stage by using video camera; **B,** checking a distinctive mark on cadaver using larvae fallen into a trap in field condition



**Figure 3-2** Number of each taxon of small mammal captured by snap traps in *Picea glehnii* plantation and natural forest the University of Tokyo Hokkaido Forest from spring 2000 to spring 2013. **A**, *Apodemus argenteus*; **B**, *Myodes rufocanus bedfordiae*; **C**, *Apodemus speciosus*; and **D**, *Sorex* spp.

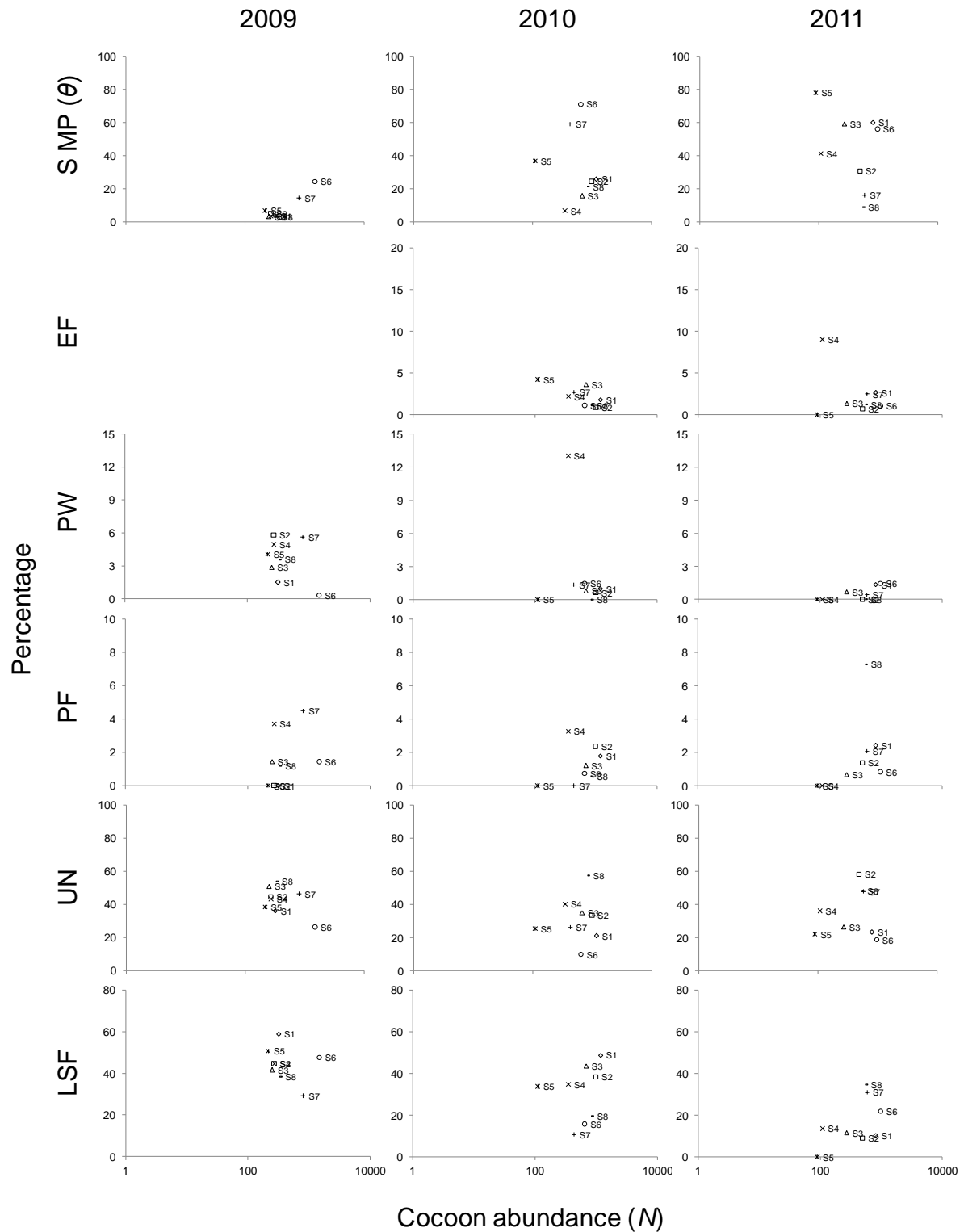


**Figure 3-3** Parasitoids of *Pristiphora erichsonii* (Hartig). **A**, *Aptesis* sp.; **B**, Tribe Mesoleiini; **C**, *Endasys* sp. (female); **D**, *Endasys* sp. (male); **E**, *Myxexoristops stolidus* (Stein); **F**, *Vibrissina turrita* (Meigen); and **G**, *Bessa parallela* (Meigen)

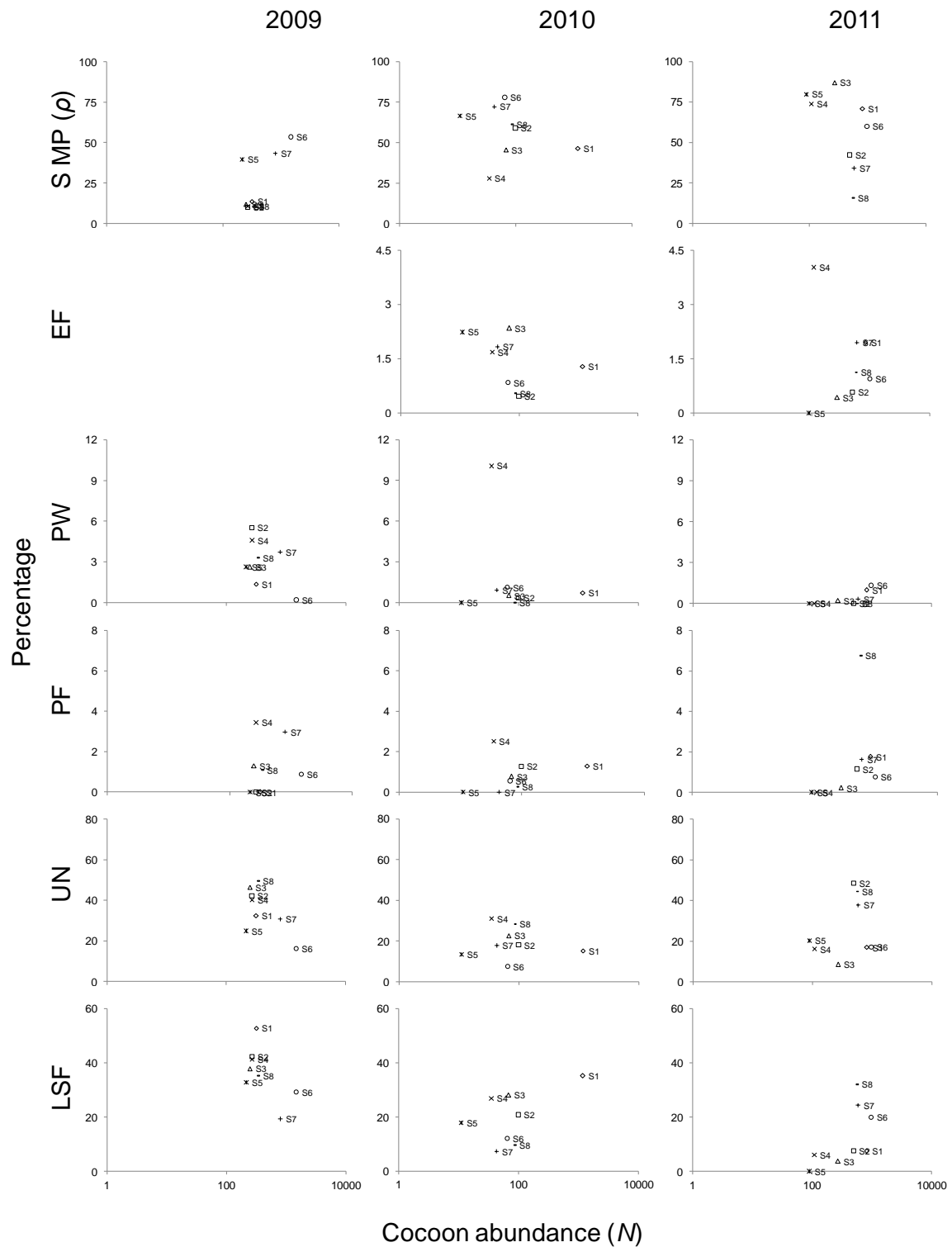


**Figure 3-4** Cocoons of *Pristiphora erichsonii* (Hartig) colonized by entomopathogenic fungi. **A**, *Beauveria* sp.; and **B**, *Isaria* sp.





**Figure 3-5** *Pristiphora erichsonii* (Hartig) and percentage of each mortality factors when small mammal predation before sampling ( $\theta$ ) was considered. Mortality (SMP, small mammal predation ( $\theta$ ); EF, entopathogenic fungi; PW, parasitic wasps; PF, parasitic flies; and UN, unknown factors) or survival (LSF; *P. erichsonii*) in each generation are shown



**Figure 3-6** *Pristiphora erichsonii* (Hartig) and percentage of each mortality factors when small mammal predation rate throughout the cocoon period ( $\rho$ ) was considered. Mortality (SMP: small mammal predation ( $\rho$ ), EF: entopathogenic fungi, PW: parasitic wasps, PF: parasitic flies, and UN: unknown factors) or survival (LSF; *P. erichsonii*) in each generation are shown

## CHAPTER 4: HOST TREE RESPONSE AGAINST HERBIVORY

### 4.1. Introduction

The physical and chemical properties of plants have been shown to influence the population dynamics of herbivores (Geri et al. 1993; Howe and Schaller 2008). Ecologically, the best-known classification of plant defenses is based on the categories of constitutive and induced defenses. Constitutive defenses do not depend on herbivore action, whereas induced defenses are activated based on herbivore activity or infection (Haukioja 2005; Karban and Baldwin 1997). Previous studies have shown that induced defenses in host plants cause population cycles in herbivores. Changes in food quality induced by previous feeding by the larch bud moth are known to have a substantial effect on the population cycle of the moth (Baltensweiler et al. 1977). Damage-induced changes in foliage quality have also been found in a sub-arctic insect herbivore (the autumnal moth)-mountain birch system (Haukioja et al. 1985).

Within stands, some host trees can be heavily infested and completely defoliated, whereas the others show only light defoliation. Plant chemistry serves primarily to defend plants against herbivores or to limit host choice by the herbivores (Hochuli 1996; Jarzomski et al. 2000). Factors that decrease the relative palatability of plants in terms of insect preferences include increases in the rigidity of the plant tissue and decreases in the water content of the foliage (Hunter and Lechowicz 1992). A low nutritional content can also serve as a mechanism of defense against herbivores if the plant tissues do not match the requirements of the herbivores (Clancy et al. 2004; Clancy et al. 1988). Sugars are commonly recognized as phagostimulants for phytophagous insects (Chapman 2003). However, the results of studies by Clancy (1992) and Clancy et al. (2003) appear to contradict this view. A wealth of literature shows that secondary compounds may be the factors that most profoundly influence herbivores (Hochuli 1996; Mattson et al. 1991). The total phenolic content of plant tissues is related to the inhibition of herbivore growth (Haukioja 2003; Rossiter et al. 1988), and condensed tannins are thought to function as antidigestives, antifeedants, toxins, and growth inhibitors (Berenbaum 1983; Feeny 1968). However, no information appears to be available on *P. erichsonii* and its interaction with its host tree.

The purpose of this chapter, based on a field investigation, was to examine the induced response of larch foliage to defoliation by *P. erichsonii*, with particular emphasis on the carbon/nutrient balance

hypothesis (CNBH). I determined the cumulative effects of successive years of defoliation by examining the difference between the response of the nitrogen concentration and that of the secondary compounds in the foliage.

## **4.2. Materials and Methods**

### **4.2.1. Needle sampling and needle properties**

Based on the defoliation intensity occurring in 2009, 2-4 sample trees were arbitrarily selected from each site excluding trees at site 4 that were not *L. kaempferi* (Fig. 1-3), including both lightly defoliated trees and heavily defoliated trees. From 2010 to 2012, in mid-July and before *P. erichsonii* defoliation, one entire branch was sampled using a bowgun because the branches were located at heights of 10-25 m. The sample branches were arbitrarily determined depending on the position of the branches and the branch density in each of the sample trees. Each of the branches was cut into 100-cm sections measured from the terminus. The mean length of the current-year long shoots (shoot length) was determined for each section. All needles on the spur shoots were then removed from the whole branch, homogenized, and randomized for freeze-drying. A total of 100 of these needles were used to determine average needle length, and another 30 needles were used to measure the dry mass individually. The remaining needles were stored in a freezer (-30 °C) in an airtight plastic bag for further chemical analysis.

### **4.2.2. Chemical analysis of needles**

Briefly explain the method of chemical analysis. Freeze-dried needles (150-200 mg, approximately 140 needles/branch) were ground to a fine powder using a vibratory ball mill and then dried completely in a vacuum drying oven for 1 day at 40 °C. The powdered needles (approximately 20 mg) were used for one measurement of each method. The concentrations of carbon (C) and nitrogen (N) and of water-soluble sugars (glucose + fructose + sucrose) and the concentrations of secondary metabolites, as represented by polyphenolics (total phenolics and condensed tannins), were determined. The concentrations of C and N for each sample were determined from one measurement with a CHNS/O Analyzer (Perkin-Elmer PE2400 Series II). Phenol-sulfuric acid reagent was used to estimate water-soluble sugar ('sugars' in the text) with a method developed by DuBois et al. (1956) and modified by Chow and Landhäusser (2004). The value was standardized against glucose. The Folin-Ciocalteu method, with tannic acid as a standard, was used

to determine total phenolics ('phenolics' in the text) (Lowman and Box 1983). The butanol-HCl method, with cyanidin as a standard, was used to quantify condensed tannins ('tannins' in the text) (Cork and Krockenberger 1991; Schofield et al. 2001). I used the mean of four replicate measurements to estimate the content of sugars and the mean of five replicate measurements to estimate the content of phenolics and of tannins. These estimates were used in the subsequent analyses. See Appendix 2 for more details.

#### **4.2.3. Adult size**

Many studies indicated that fecundity was strongly influenced by adult body size especially for species of which adults do not take food (e.g. Jervis et al. 2003; Marshall 1990). Length of forewing is being used as body size parameter because size of wing does not change after emergence. In this study, only females were used for measuring length of forewing using a digital vernier caliper.

#### **4.2.4. Statistical analysis**

The effects of *P. erichsonii* defoliation on the chemical and physical properties of foliage (carbon and nitrogen concentration, phenolics, tannins, sugars, needle length, needle dry mass, shoot length) were determined. Based on the 2009 defoliation intensity (DF09), all sample trees were classified into two groups. Group 1 included trees with light defoliation ( $\leq 20\%$  defoliation intensity), and Group 2 included those with severe defoliation ( $\geq 70\%$  defoliation intensity). The annual change in the foliage properties was compared between the two groups.

A linear model (LM) was employed to determine the effect of each year of defoliation intensity on the foliage properties in each year. The defoliation intensity was arcsine transformed before the analysis. The tests involved only the defoliation intensity before the foliage was sampled.

The LM and a linear mixed model (LMM) were employed to determine the effects of defoliation and of the site on the foliage properties. The foliage properties were used as dependent variables. The data from 3 years (2010-2012) were combined for analysis. The defoliation intensity in 2009 (DF09 or Group 1-2) and year were employed as independent variables in the LM and fixed effects in the LMM. The values of DF09 were arcsine transformed before the analysis. The intensity of defoliation in 2010-2012 was not included as an independent variable because the intensity of future defoliation did not influence the foliage properties in the past, e.g., the intensity of defoliation in 2011 did not influence the foliage properties in

2009-2011. Because the variance in defoliation intensity among individual trees was small in 2010 (DF10) and 2011 (DF11), the year was included as an independent variable. The sites were modeled as a random effect in the LMM. The Akaike information criterion (AIC) was employed to select the best model. The difference in the AIC between the best model and a null model ( $\Delta AIC$ ) was also calculated to test the significance of the best model. If the LMM was selected as the best model, it was concluded that the foliage property used as the dependent variable in the model shown a site effect. If a model with DF09 was selected as the best model and the effect of DF09 was significant, it was concluded that the foliage property was influenced by the 2009 defoliation intensity. If a model with the group was selected and the effect of the group was significant, it was concluded that the effect of the 2009 defoliation intensity was also significant but was weaker than in the previous case.

In each site, the LM was employed to test the influence of year to length of forewing. The length of 2009 was compared with that of 2010 and 2011. The length of 2010 was compared with that of 2011 and the length of 2011 was compared with that of 2012, respectively.

All statistical analyses were conducted with R 2.14.2 statistical software (R Development Core Team 2012). The 'nlme' library was used for the LMM (Pinheiro et al. 2013).

### **4.3. Results**

#### **4.3.1. Needle properties**

The results for the measured properties, including the defoliation intensity of the sample trees for these analyses, are shown in the Appendix 3.

Figure 4-1 shows the annual changes in chemical properties for the two groups. Significant differences between the two groups were found in phenolics (2010), nitrogen (2010), and the CN ratio (2010) ( $P < 0.01$ ,  $t$ -test) and tannins (2010), carbon (2011), and the CN ratio (2011) ( $P < 0.05$ ,  $t$ -test). The difference in chemical properties was marginally significant for nitrogen (2011) and sugars (2012) ( $P < 0.1$ ,  $t$ -test) (Fig. 4-1). Nitrogen decreased linearly with year, whereas other properties tended to increase. In 2010, nitrogen was greater in Group 1 than in Group 2 (Fig. 4-1E), whereas the concentrations of phenolics and tannins and the CN ratio were greater in Group 2 than in Group 1 (Fig. 4-1A, B, F). The concentration of phenolics in both groups and tannins in Group 2 did not differ between 2011 and 2012 (Fig. 4-1A, B). The concentration of tannins in Group 1 increased from 2011 to 2012 and reached that of

Group 2 in 2012 (Fig. 4-1B). Similarly, nitrogen and the CN ratio in Group 1 reached the values for Group 2 (Fig. 4-1E, F). Sugars continued to increase until 2012, although no significant difference was found between the two groups in all 3 years (Fig. 4-1C). Carbon also increased but did so in a different manner in the two groups (Fig. 4-1D).

Figure 4-2 shows the annual changes in the physical properties of the two groups. Needle length was lower in Group 2 than in Group 1 and gradually decreased until 2012 (Fig. 4-2A). The difference in needle length between the two groups was marginally significant only in 2010 (Fig. 4-2A) ( $P < 0.1$ ,  $t$ -test). Needle dry mass was less in Group 2, but not significantly so ( $P > 0.1$ ,  $t$ -test), and decreased in 2012 (Fig. 4-2B). Shoot length tended to decrease until 2012, with no consistent difference between the two groups (Fig. 4-2C).

Table 4-1 shows the results of the LM used to determine the cumulative effects of defoliation on foliage properties. DF09 significantly influenced many properties in 2010 and 2011: nitrogen (2010 and 2011), the CN ratio (2010 and 2011) ( $P < 0.01$ ); phenolics (2010 and 2011), carbon (2011), needle length (2010) ( $P < 0.05$ ); tannins (2010 and 2011), sugar (2011), and needle length (2011) ( $P < 0.1$ ). However, DF10 and DF11 had fewer significant effects: DF10 on nitrogen (2011,  $P < 0.1$ , and 2012,  $P < 0.05$ ), on the CN ratio (2011,  $P < 0.1$ , and 2012,  $P < 0.05$ ), and DF11 on needle dry mass (2012,  $P < 0.01$ ) and on shoot length (2012,  $P < 0.1$ ).

Table 4-2 shows the best model (LM or LMM) for determining the influences of *P. erichsonii* defoliation and of sites. The effects of defoliation (DF09 or group) were significant for phenolics, nitrogen, the CN ratio ( $P < 0.01$ ), tannins, and needle length ( $P < 0.05$ ) and marginally significant for sugars and needle dry mass ( $P < 0.1$ ). The LMM was selected as the best model for phenolics, sugars, and the CN ratio. The effect of the year 2012 was significant ( $P < 0.01$ ) for all properties except needle dry mass. The effect of the year 2011 was significant for phenolics and sugars ( $P < 0.01$ ).

#### 4.3.2. Adult size

Length of female forewing tended to reduce from generation 2009 to generation 2012 (Fig. 4-3). The size was greatest in the generation 2009 and smallest in the generation 2012 with small variation among sites, whereas the size of generation 2010 was intermediate with great variation among sites. Within-site

variation in size was small for generation 2009 but greater for generations 2010 (site 5, 6, and 7) and 2011 (site 2 and 3). From generations 2009 to 2010, the mean size reduced most greatly at site 5 followed by sites 6 and 7 with significant difference (LM,  $P < 0.05$ ). At sites 1, 2, and 8, significant difference was not found between generations 2009 and 2010 but between generations 2010 and 2011 (LM,  $P < 0.05$ ) because the size reduced greatly in 2011. In 2012, the mean size reduced greatly at all sites. Significant difference was found between 2011 and 2012 at a significant level of  $P < 0.01$  at sites 6, 7, and 8, and at a significant level of  $P < 0.05$  at sites 1 and 2 (LM).

#### 4.4. Discussion

Several previous studies have shown that defoliation produced by insects or by artificial procedures can affect the phytochemical composition of the foliage. This change in composition is expected to decrease the preference and performance of insect herbivores (Geri et al. 1993; Haukioja 2005; Wagner and Evans 1985). In particular, the carbon/nutrient balance hypothesis (CNBH) implies the occurrence of a nutrient deficiency and a resulting accumulation of carbon-based secondary compounds or carbohydrate storage (Bryant et al. 1983; Karban and Myers 1989; Stamp 2003). In this study, as expected, the nitrogen concentration decreased linearly after successive years of *P. erichsonii* defoliation (Fig. 4-1E), whereas phenolics, tannins, and the CN ratio responded in the opposite manner (Fig. 4-1A, B, F). Insect defoliation is known to cause both carbon and nitrogen plant deficits (Bryant et al. 1991; Haukioja et al. 1985).

Nitrogen concentration was strongly influenced by the past defoliation intensity, whereas carbon was not (Tables 4-1A, 4-2A). However, needle length and needle dry mass were significantly related to the defoliation intensity (Table 4-2B), most likely because these properties were related to the carbon deficit before bud flushing in the year following severe defoliation. In contrast, the carbon concentration in the foliage, measured in July, was also influenced by photosynthesis after bud flushing during the current year (Bryant et al. 1983). Most likely, this additional influence of the current year needle was the reason that significant effects of the specific year (s) of past defoliation intensity on the carbon concentration were not found (Tables 4-1A, 4-2A). Shoot length decreased with year, most likely due to successive years of severe defoliation by *P. erichsonii* (Fig. 4-2C). However, significant effects of the specific year (s) of past defoliation intensity on the shoot length were not found (Tables 4-1B, 4-2B), most likely for the same reason. Some long shoots flush at the same time as the spur shoots but other long shoots begin to flush



later and continue to elongate until early summer (Panisara Pinkantayong, personal observation). For this reason, it is probable that the shoot length, at least latter case, was influenced by photosynthesis in the current year. Phenolics, tannins, and the CN ratio were, most likely, up-regulated under nitrogen limitation in the year following severe defoliation in a way similar to that found by many previous studies (e.g. Bryant et al. 1991; Roitto et al. 2009). The decrease in nitrogen and increase in the CN ratio continued until 2012. However, the concentrations of the secondary metabolites (phenolics and tannins) in 2011 and 2012 did not differ, with the exception of tannins in Group 1 (Fig.4-1). This result implies that limitations affected the syntheses of plant secondary metabolites.

Kamata et al. (1996) conducted a manual defoliation experiment for two consecutive years and found that the effect of delayed induced response was cumulative. In this study, many foliar properties were significantly influenced by DF09. However, fewer foliage properties were affected by DF10 or DF11 (Table 4-1), most likely because the variation in defoliation intensity among trees was high in 2009 but low in 2010 and 2011 (Fig. 2-5). These results suggest that the past defoliation history additively affected the foliage properties in the 2 years following the insect defoliation. However, as the significant effects of DF09 disappeared in 2012, this 'stamp effect' tended to be weak with time and disappeared in 3 years.

Nitrogen, a regulator of many secondary metabolites, and needle length and dry mass, which were directly influenced by the carbon deficit before bud flushing, were not influenced by sites but only by defoliation intensity, based on the finding that the LM was selected as the best model (Table 4-2). In contrast, phenolics, sugars, and the CN ratio were influenced by sites, based on the finding that the LMM was selected (Table 4-2). In terms of a site effect on phenolics and sugars, as indicated by a random effect in the LMM, the value (intercept) was greatest for site 6 but smallest for site 7 (Table 4-3), although all sample trees at both sites 6 and 7 were severely defoliated in 2009 and categorized in Group 2 with one exception (tree No. 304, Appendix3). This finding is a direct result of the site effects for phenolics and for sugars. The values of the random effects for phenolics and sugars (Table 4-3) indicate that the concentrations of phenolics and sugars were high at site 6 but low at site 7. A site effect on the CN ratio was also found but occurred in a different manner. The value of the random effect for the CN ratio was highest at site 6 and next highest at site 7, indicating that the CN ratio was high at both sites. This inconsistency between the site effect on the CN ratio and the site effect on phenolics and sugars also

supports the hypothesis that the synthesis of phenolics was most likely regulated not only by nitrogen limitation but also by other factors. The trees at site 7 may have experienced environmental stress from a source other than *P. erichsonii* defoliation, and this alternative stress would have decreased the concentration of phenolics and sugar. However, it cannot definitively identify the source of stress at site 7, and further studies are needed.

The reduction of female body size is believed to be related the fecundity especially for species of which adults do not take food (Honek 1993). Fecundity changed greatly with body size in the beech caterpillar moth, *Syntypistis* (= *Quadricalcarifera*) *punctatella* (Kamata et al. 1996). Reduced potential fecundity related body size can depress population density by reducing initial number of the next generation (Dempster and Pollard 1981). Actually female fecundity of *P. erichsonii* was reported to decrease with year during outbreaks (Ives 1976). In this study, adult body size (measured female forewing length) of *P. erichsonii* tended to reduce from generation 2009 to generation 2012 (Fig. 4-3). Two possible causes of the reduction in body size during population outbreaks were food shortage and food deterioration. Starvation in larval stage during high density period is known to cause high mortality and reduction in body size (Hard 1971). Food shortage occurred at the earlier stage of the instar V when the larval density extremely high. As a result, a fewer individuals could survive and spin cocoons at higher larval density (Fig. 2-10). Food deterioration caused by combination of an enhanced chemical defense triggered by defoliation (Roland and Myers 1987; Schultz and Baldwin 1982), reduction in nitrogen concentration (Clancy et al. 1988; Fox and Macauley 1977; Myers and Post 1981; Scriber and Slansky 1981), or increased fiber concentration (Baltensweiler et al. 1977; Baltensweiler and Fischlin 1988), is also considered to reduce insect body size (see Karban and Myers 1989). On the other hand, there were many studies on sawflies on pine trees, in which neither significant reduction in larval performance nor growth were found in the year following severe insect defoliation (e.g. Niemelä et al. 1991; Niemelä et al. 1984), or even improved performance with increase in prior defoliation (Raffa et al. 1998). These are, however, the cases of sawfly feeding on evergreen conifer (*Pinus* spp.). Evergreen conifers differ from deciduous species in their lack of major storage reserves in stem and root (Chapin et al. 1990; Kozłowski and Keller 1966). Scots pine, *Pinus sylvestris*, retains needles for three seasons (Heimann and Pellitteri 2006). Old foliage on evergreen woody plants is assumed to play a major role in improving the carbon balance in

supplying carbon to new sprouting foliage (Chapin et al. 1990; Jonasson 1995) so that evergreen conifers suffer more severe carbon deficit than deciduous species by severe insect defoliation that defoliates old foliage as well as current-year foliage. Regarding the evergreen conifers, new foliage become less defensive in the year following the severe defoliation by the carbon deficit following carbon/nutrient balance hypothesis (Bryant et al. 1983), which improve host suitability for insect herbivores. However, *P. erichsonii* feeds on a deciduous conifer so the response of plant and resulted insect performance and growth in the year following severe defoliation were similar to those reported in many folivorous insects feeds on deciduous trees. In present study, body size of generation 2010 and 2011 varied greatly within the sites (sites 5, 6, and 7 for the generation 2010 and sites 2 and 3 for the generation 2011). Great variance in body size within each site was likely due to food quality and food shortage. As shown in the Table 4-1, foliage quality was strongly influenced by DF09. The DF09 varied greatly at sites 6 and 7 compared to other sites (Fig. 2-6). However, variation was smaller in DF09 of sites 2 and 3 than in those of sites 6 and 7. Furthermore, at site 2, variation in DF10 was small. Therefore, great variation in body size does not seem to be caused by only variation in foliage quality. On the other hand, many larvae were crawling on tree trunks after the foliage was almost lost in the site 6 and 7 in 2010 and site 5 in 2011 (Panisara Pinkantayong, personal observation). The number of newly spun cocoons (*N*) at sites 2 and 5 in 2011 was much smaller than that have been expected from abundance of instar IV (Fig. 2-10). The number of cocoons at sites 6 and 7 in 2010 was also smaller than other sites despite of more intense defoliation at the two sites. Food shortage was a likely cause of these relatively smaller numbers of cocoons compared to high larval densities. Furthermore, the cocoons of generation 2011 could not survive at all after overwintering at site 5 (Table 2-5). It is noteworthy that the number of cocoons decreased when the larval density was higher than a certain threshold (Fig 2-10). Smaller numbers of larvae could mature before food shortage because greater numbers of young larvae had fed on the limited food resource.

Regarding the generations 2009-2011, both food deterioration and food shortage were related to the reduction in body size but it is impossible to separate those two effects in this study. However, fortunately, no complete defoliation was observed in any trees at the eight sites in 2012 so all the reduction in size can be attributed to food deterioration. Interestingly, the reduction was greatest in generation 2012. In 2012, nitrogen concentration continued to decrease whereas tannins and phenolics stopped to increase.

Therefore response of *P. erichsonii* body size to food deterioration is likely non-linear suggesting some influences such a maternal effect through generations.

**Table 4-1** The results of a linear model to test the influence of past defoliation by the larch sawfly, *Pristiphora erichsonii* (Hartig), on foliage properties of the Japanese larch, *Larix kaempferi* (Lamb.) Carr.

**A**, chemical properties; **B**, physical properties

(A) Chemical Property	Independent variable	Coefficient	<i>P</i>	ΔAIC	(B) Physical Property	Independent variable	Coefficient	<i>P</i>	ΔAIC
% Phenolics 2010	DF09	0.23x10 <sup>1</sup>	0.02*	4.78	Needle length 2010	DF09	-2.73x10 <sup>-1</sup>	0.03*	3.27
% Phenolics 2011	DF09	0.26x10 <sup>1</sup>	0.04*	3.19	Needle length 2011	DF09	-3.39x10 <sup>-2</sup>	0.06†	2.26
	DF10	0.26x10 <sup>1</sup>	0.12	0.92		DF10	-4.96x10 <sup>-2</sup>	0.84	-1.95
% Phenolics 2012	DF09	4.70x10 <sup>-1</sup>	0.64	1.52	Needle length 2012	DF09	-2.62x10 <sup>-2</sup>	0.89	-0.98
	DF10	0.21x10 <sup>1</sup>	0.24	9.97		DF10	-2.78x10 <sup>-1</sup>	0.39	1.43
	DF11	3.56x10 <sup>-1</sup>	0.82	-1.94		DF11	-4.51x10 <sup>-1</sup>	0.12	0.88
% Tannins 2010	DF09	5.42x10 <sup>-1</sup>	0.07†	1.98	Needle dry mass 2010	DF09	-1.65x10 <sup>-1</sup>	0.41	-1.19
% Tannins 2011	DF09	0.10x10 <sup>1</sup>	0.10†	1.36	Needle dry mass 2011	DF09	-1.06x10 <sup>-1</sup>	0.51	-1.47
	DF10	0.10x10 <sup>1</sup>	0.20	0.04		DF10	1.93x10 <sup>-2</sup>	0.93	-1.99
% Tannins 2012	DF09	-3.61x10 <sup>-1</sup>	0.50	0.71	Needle dry mass 2012	DF09	8.56x10 <sup>-2</sup>	0.54	-1.31
	DF10	7.06x10 <sup>-1</sup>	0.45	5.81		DF10	-1.59x10 <sup>-1</sup>	0.50	-0.91
	DF11	0.10x10 <sup>1</sup>	0.21	-0.12		DF11	-5.67x10 <sup>-1</sup>	<0.01**	8.62
% Sugar 2010	DF09	8.24x10 <sup>-1</sup>	0.40	-1.17	Shoot length 2010	DF09	-5.17x10 <sup>-2</sup>	0.96	-2.00
% Sugar 2011	DF09	0.24x10 <sup>1</sup>	0.08†	1.72	Shoot length 2011	DF09	-2.28x10 <sup>-1</sup>	0.69	-1.82
	DF10	0.16x10 <sup>1</sup>	0.37	-1.03		DF10	-0.13x10 <sup>1</sup>	0.12	0.84
% Sugar 2012	DF09	9.20x10 <sup>-1</sup>	0.19	2.99	Shoot length 2012	DF09	6.38x10 <sup>-1</sup>	0.45	-1.33
	DF10	0.14x10 <sup>1</sup>	0.22	9.44		DF10	3.54x10 <sup>-1</sup>	0.75	-1.88
	DF11	-4.67x10 <sup>-1</sup>	0.67	-1.79		DF11	-0.21x10 <sup>1</sup>	0.06†	2.08
% Carbon 2010	DF09	-2.10x10 <sup>-1</sup>	0.41	-1.21					
% Carbon 2011	DF09	6.03x10 <sup>-1</sup>	0.04*	2.92					
	DF10	5.30x10 <sup>-1</sup>	0.19	0.13					
% Carbon 2012	DF09	1.10x10 <sup>-1</sup>	0.59	-0.30					
	DF10	4.21x10 <sup>-1</sup>	0.21	2.96					
	DF11	-1.60x10 <sup>-1</sup>	0.62	-1.71					
% Nitrogen 2010	DF09	-3.92x10 <sup>-1</sup>	<0.01**	6.52					
% Nitrogen 2011	DF09	-2.17x10 <sup>-1</sup>	<0.01**	7.60					
	DF10	-2.02x10 <sup>-1</sup>	0.06†	2.35					
% Nitrogen 2012	DF09	-7.47x10 <sup>-2</sup>	0.24	-2.26					
	DF10	-2.20x10 <sup>-1</sup>	0.03*	-2.27					
	DF11	-2.17x10 <sup>-2</sup>	0.83	-1.95					
CN ratio 2010	DF09	0.53x10 <sup>1</sup>	<0.01**	8.05					
CN ratio 2011	DF09	0.50x10 <sup>1</sup>	<0.01**	11.28					
	DF10	0.41x10 <sup>1</sup>	0.07†	1.97					
CN ratio 2012	DF09	0.23x10 <sup>1</sup>	0.21	4.64					
	DF10	0.63x10 <sup>1</sup>	0.04*	17.49					
	DF11	1.79x10 <sup>-1</sup>	0.95	-2.00					

\*\* *P* < 0.01, \* *P* < 0.05, †, *P* < 0.1

**Table 4-2** The best model of the effects of *Pristiphora erichsonii* (Hartig) defoliation in 2009 (DF09 or group) and of sites on foliage properties of *Larix kaempferi* (Lamb.) Carr. using a linear model and a linear mixed model. **A**, chemical properties; and **B**, physical properties

(A)Chemical Property	Independent factor	Coefficient	P	(B)Physical Property	Independent factor	Coefficient	P
Phenolics (%)	(Intercept)	0.93x10 <sup>1</sup>	<0.01**	Needle length	(Intercept)	0.23x10 <sup>1</sup>	<0.01**
(LMM)	Group 2	0.27x10 <sup>1</sup>	<0.01**	(LM)	Group 2		
ΔAIC=31.88	Year2011	0.40x10 <sup>1</sup>	<0.01**	ΔAIC=20.55	Year2011	2.67x10 <sup>-2</sup>	0.86
	Year2012	0.32x10 <sup>1</sup>	<0.01**		Year2012	-4.04x10 <sup>-1</sup>	<0.01**
	DF09				DF09	-2.67x10 <sup>-1</sup>	0.02*
Tannins (%)	(Intercept)	0.23x10 <sup>1</sup>	<0.01**	Needle dry mass	(Intercept)	0.15x10 <sup>1</sup>	<0.01**
(LM)	Group 2			(LM)	Group 2		
ΔAIC=23.59	Year2011	6.95x10 <sup>-1</sup>	0.10	ΔAIC=7.62	Year2011	2.48x10 <sup>-1</sup>	0.10
	Year2012	0.15x10 <sup>1</sup>	<0.01**		Year2012	-1.44x10 <sup>-1</sup>	0.28
	DF09	7.55x10 <sup>-1</sup>	0.02*		DF09	-2.18x10 <sup>-1</sup>	0.05†
Sugar (%)	(Intercept)	1.31x10 <sup>1</sup>	<0.01**	Shoot length	(Intercept)	0.92x10 <sup>1</sup>	<0.01**
(LMM)	Group 2	0.17x10 <sup>1</sup>	0.06†	(LM)	Group 2		
ΔAIC=35.68	Year2011	0.17x10 <sup>1</sup>	<0.01**	ΔAIC=29.73	Year2010	-5.10x10 <sup>-1</sup>	0.42
	Year2012	0.44x10 <sup>1</sup>	<0.01**		Year2011	-0.30x10 <sup>1</sup>	<0.01**
	DF09				Year2012	-0.33x10 <sup>1</sup>	<0.01**
Carbon (%)	(Intercept)	4.71x10 <sup>1</sup>	<0.01**		DF09		
(LM)	Group 2						
ΔAIC=25.70	Year2011	3.06x10 <sup>-1</sup>	0.10				
	Year2012	0.10x10 <sup>1</sup>	<0.01**				
	DF09						
Nitrogen (%)	(Intercept)	0.20x10 <sup>1</sup>	<0.01**				
(LM)	Group 2						
ΔAIC=44.61	Year2011	-8.19x10 <sup>-2</sup>	0.38				
	Year2012	-4.11x10 <sup>-1</sup>	<0.01**				
	DF09	-2.69x10 <sup>-1</sup>	<0.01**				
CN ratio	(Intercept)	2.38x10 <sup>1</sup>	<0.01**				
(LMM)	Group 2						
ΔAIC=57.43	Year2011	0.16x10 <sup>1</sup>	0.30				
	Year2012	0.90x10 <sup>1</sup>	<0.01**				
	DF09	0.33x10 <sup>1</sup>	<0.01**				

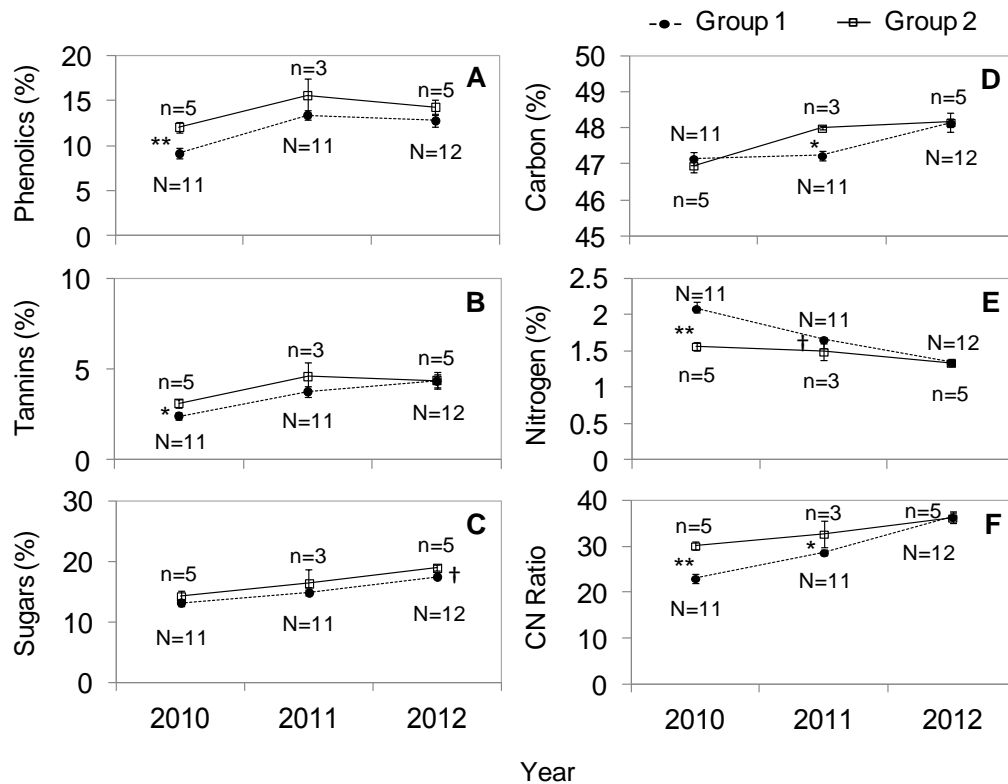
The results from the best model are shown

\*\*  $P < 0.01$ , \*  $P < 0.05$ , †,  $P < 0.1$

**Table 4-3** Effects of sites on total phenolics, water-soluble sugars, and the CN ratio of the Japanese larch, *Larix kaempferi* (Lamb.) Carr., indicated by a random effect (intercept) obtained from a linear mixed model

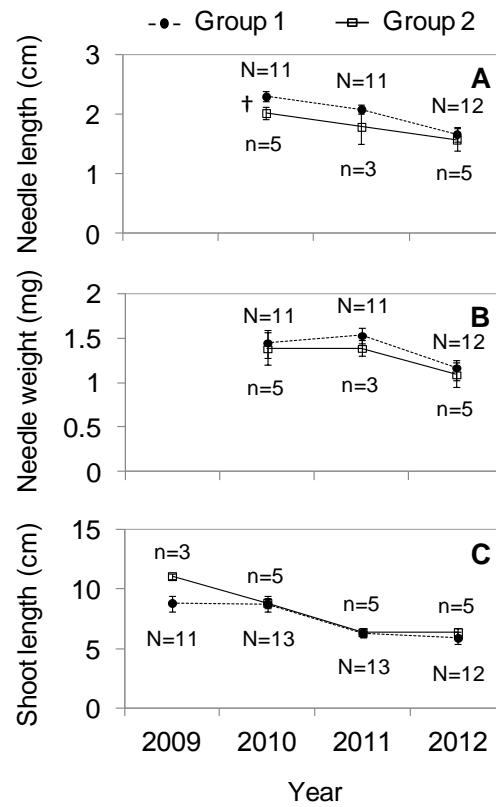
Site	Random effect (intercept)		
	Phenolics	Sugars	CN ratio
1	0.0514	0.8359	-0.0432
2	-0.0447	0.0550	-0.7037
3	0.3326	-0.5060	0.0201
5	-0.4131	-0.1008	-1.0768
6	0.8133	1.1694	0.7490
7	-0.8137	-0.8486	0.6545
8	0.0743	-0.6050	0.4001

Site 4 was not included in this table because larch specie other than *L. kaempferi* were planted

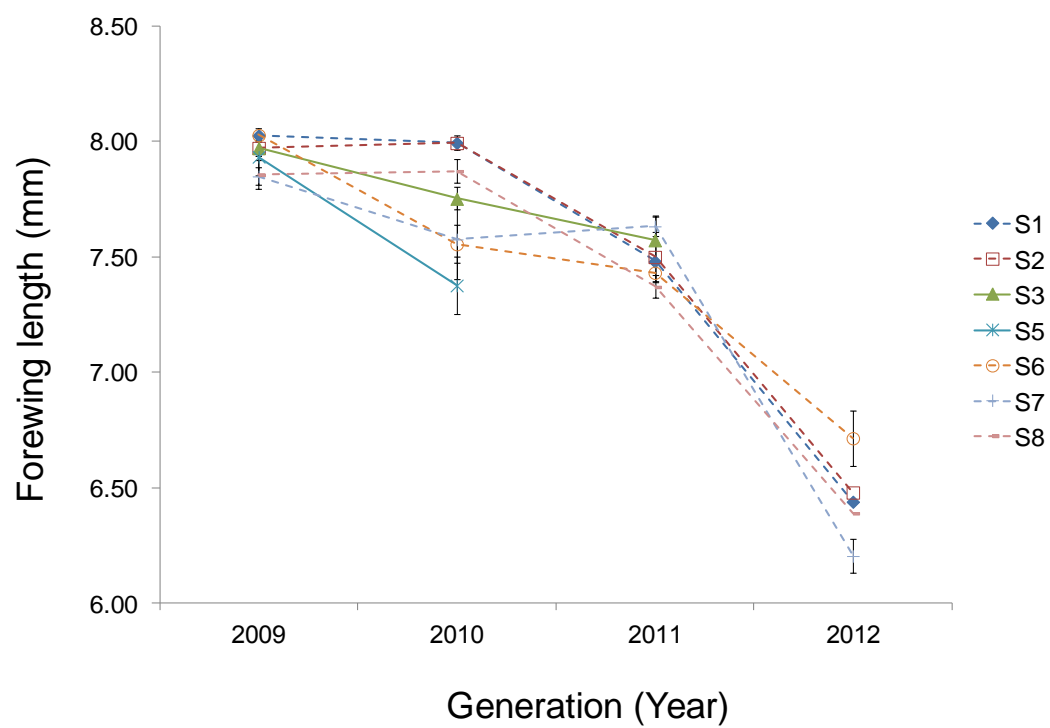


**Figure 4-1** Annual changes in chemical properties of Japanese larch, *Larix kaempferi* (Lamb.) Carr., needles in the years following insect defoliation. **A**, Total phenolics (%); **B**, condensed tannins (%); **C**, water-soluble sugars (%); **D**, carbon (%); **E**, nitrogen (%); and **F**, CN ratio. All sample trees were categorized into Groups 1 or 2 depending on defoliation intensity in 2009: Group 1, light defoliation ( $\leq 20\%$  defoliation intensity); Group 2, severe defoliation ( $\geq 70\%$  defoliation intensity). *N*, the number of sample trees in Group 1; *n*, the number of sample trees in Group 2. Bar  $\pm$ SE. Statistical significance: \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; †,  $P < 0.1$





**Figure 4-2** Annual changes in physical properties of Japanese larch, *Larix kaempferi* (Lamb.) Carr., needles in the years following insect defoliation. **A**, Needle length (cm); **B**, needle dry mass (mg); and **C**, shoot length (cm). All sample trees were categorized into Groups 1 or 2 depending on defoliation intensity in 2009: Group 1, light defoliation ( $\leq 20\%$  defoliation intensity); Group 2, severe defoliation ( $\geq 70\%$  defoliation intensity).  $N$ , the number of sample trees in Group 1;  $n$ , the number of sample trees in Group 2.  $\text{Bar} \pm \text{SE}$ . Statistical significance: \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; †,  $P < 0.1$



**Figure 4-3** Length of female forewing from generation 2009 to generation 2012 (mean  $\pm$  SE)

## CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

A broad question of this study was why *P. erichsonii*'s population outbreaks continue so long? In Hokkaido (Japan), the *P. erichsonii* exhibited several distinctive population patterns. Population outbreaks certainly depended on scales: At the regional scale, it lasts almost 10 years or more. At a landscape scale, it lasts almost 5 years or more, and at a stand level, a 5-year outbreak represented only in a small epicenter (sustained eruption) and 2-year outbreaks at most stands (pulse eruption). The intermediate pattern between sustained eruption and pulse eruption was also found at one of all research sites. In this study, site 7 and its surroundings were an epicenter of severe defoliation in 2009 and spread out thereafter. Spatial distribution of sustained type and pulse type of eruption is not a dichotomy but a continuum changing gradually from an epicenter to peripherals. As a result, medium and light areas of defoliation extending site 6 and the rest stands. In 2012, the defoliated area shrank to small area around the epicenter (site 7), where severe defoliation have still continued. This result indicates that conditions at site 7 and its surroundings were more favorable to *P. erichsonii* than the other stands. Root (1973) proposed the resource-concentration hypothesis that herbivores, especially dietary specialists, are more likely to find and remain on host plants growing in dense pure stands and thus achieve higher equilibrium densities there. The resource concentration hypothesis can explain the reason why site 7 and its surroundings could be an epicenter of *P. erichsonii* outbreaks because many larch plantations were concentrated round the site 7 (Fig. 1-3). At other six stands, severe defoliation was observed only 2 years (2010-2011). Density of *P. erichsonii* decreased in 2012 as shown by this study and in 2013 according to a personal communication by Ryo Hotta although no natural enemies regulated the population outbreaks similarly to the epicenter. Furthermore, newly spun cocoons in the fall of 2013 and adults emerging from the cocoon in the summer of 2014 decreased greatly (Ryo Hotta, personal communication). There are some reports revealed that *P. erichsonii* outbreaks were terminated by climatic catastrophes or host plant deterioration (Drouin et al. 1968; Lejeune et al. 1955). However, no climatic catastrophes were observed commonly in all the eight research sites.

The results of this study also support no natural enemies regulated *P. erichsonii* outbreaks. Parasites, including parasitoids and entomopathogenic fungi, did not operate effectively during a cocoon stage. The percentages of mortality caused by each of these parasite groups were smaller than 15%. No

strong density dependence was found in those of factors. The unknown mortality (UN), which was greater than those of identified parasites but smaller than 60%, had no density dependence or tendency to increase with year. These results coincided with former reports (Ives 1981; Krause and Raffa 1996; Muldrew 1956). Tachibana and Nishiguchi (1984) speculated that “small mammals predate on cocoons parasitized by parasitic wasps, which lower the parasite density and make numerical response of the parasite slow”. Furthermore, the density dependence appeared to exist between parasitic wasp of *P. erichsonii* and its hyperparasite making numerical response of the parasite slow (Ives 1976). However, asynchrony of parasitoids with life cycle of *P. erichsonii* was a more likely cause of low percentage of parasitism because they absolutely needed alternative hosts to parasitize *P. erichsonii* larvae. Cocoon fibers itself was likely to act as defense against infection by entomopathogenic fungi (Danks 2004). *P. erichsonii* larvae are also likely resistant to fungal infection because both by artificial dissemination of *B. bassiana* spores undefiled condition and by artificial spray onto the free-living larvae, the infection rate of *B. bassiana* to larvae was low (MacLeod and Heimpel 1955).

No effective predators were found during free-living larval stage. Since larvae of sawflies have protective behaviors. Nematine larvae expose their abdominal glands by rearing abdomen quickly in a snap-bend as warning signal (Jonsson et al. 1988) and produce defensive liquid from the ventral glands against predators and parasitoids (Prop 1960; Smith 1993). This liquid contains oxidized monoterpenes originating from host monoterpenes. These compounds not only deter predators but also act as an alarm (Geri et al. 1993), which do not appear to be common in other insect defoliators, such as lepidopteran species, although the “snap-bend pause” occur in the lepidopteran family Notodontidae. Furthermore, the sawfly larvae have immunity to encapsulate eggs of parasitoid by phagocytic blood cells (Muldrew 1953).

In 2012 and 2013, conspicuous defoliation was observed only at site 7 and its surroundings (Ryo Hotta, personal communication). Newly spun cocoons in the fall of 2013 and adults emerging from the cocoon in the summer of 2014 decreased greatly (Ryo Hotta, personal communication). Two biotic factors, small-mammal predation and food deterioration, were likely causes of the population decline. The percentage of small-mammal predation increased with year, even in the fall 2013 (Ryo Hotta, personal communication), however, the small-mammal predation acted in inversely density-dependent manner spatially, which would be resulted from a well-known type III functional response (Holling 1959). The

small-mammal trapping data indicated that a great number of *P. erichsonii* cocoons supported winter population densities of the three major small mammal species higher than those before the outbreak. On the contrary to gradual increase of predation rate by the small mammals, the trap catches of small mammals declined gradually with decrease of *P. erichsonii* population. Therefore, functional response in the small mammal predation was as a likely cause of gradual increase of the predation rate with year. However, a mechanism of the functional response is not clear. In general, natural enemies without a numerical response cannot regulate population outbreaks of insects. Actually the percentage of small mammal predation was no greater than 90% so the small mammal predation could not independently depress population density because the percentage of mortality to cause population decline should be greater than 97.2% under conditions that female fecundity is 38.1 (Higashiura 1988) and a proportion of female is 0.95. Food shortage and food deterioration caused great mortality before spinning cocoons and reduction of body size. However, mortality before spinning cocoons could not terminate population outbreaks because population density sometimes increased again in the following year. Gradual reduction in body size, which resulted in reduction in fecundity (Ives 1976), seemed a likely pathway of food deterioration to reduce population density of *P. erichsonii*. A reduction of fecundity can depress population density by reducing initial number of the next generation (Dempster and Pollard 1981).

At present attempts to determine the factor responsible for the termination of *P. erichsonii* outbreaks have not been successful yet. At an epicenter, a lack of quick and strong density dependent mortality factors is a direct cause of prolonged population outbreaks of *P. erichsonii* as well as a large patches with high density of host plants. In most of larch stands, *P. erichsonii* outbreaks continued for two years. The decline of population has been either attributed to food deterioration and gradual increase of small mammal predation. However, catastrophes, which include climatic or host plant catastrophes, may be needed for termination of *P. erichsonii* outbreaks at a landscape level.

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## APPENDICES

**Appendix 1** Life table for the 2009-2012 generation of *Pristiphora erichsonii* (Hartig) at eight research sites in the University of Tokyo Hokkaido Forest, Japan

	Number alive/ ((X)/m <sup>2</sup> )							
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
<b>Generation 2009</b>								
Cocoon ( <i>N</i> )	316.8	268.3	248.8	269.5	215.0	1477.5	798.0	330.5
Cocoon ( <i>I</i> )	274.1	241.6	219.3	241.3	129.9	686.5	452.8	295.2
Adult ( <i>A</i> )	167.0	113.4	94.2	111.4	70.6	431.6	154.2	116.6
Female (proportion of ♀ generation 2009 = <b>0.98</b> )	163.7	111.1	92.3	109.1	69.2	423.0	151.1	114.3
<b>Generation 2010</b>								
Egg estimated by female density x fecundity (38.1)	6236.8	4234.0	3518.1	4158.5	2636.0	16115.3	5758.8	4354.8
Cocoon ( <i>N</i> )	1174.3	984.8	679.3	350.3	110.3	647.0	428.8	833.0
Cocoon ( <i>I</i> )	628.8	403.4	369.4	252.6	37.0	142.9	119.5	323.2
Adult ( <i>A</i> )	412.5	204.6	190.9	94.0	19.7	77.8	31.3	80.5
Female (proportion of ♀ generation 2010 = <b>0.93</b> )	383.6	190.3	177.5	87.4	18.3	72.3	29.1	74.9
<b>Generation 2011</b>								
Egg estimated by female density x fecundity (38.1)	14614.9	7248.9	6764.4	3330.6	698.6	2755.2	1110.4	2852.7
Larval								
Instar I	1805.0	2734.0	1026.0	258.0	2197.0	676.0	386.0	1044.0
Instar II	1956.0	2941.0	1037.0	327.0	2227.0	709.0	514.0	1209.0
Instar III	1277.0	2218.0	729.0	332.0	1797.0	705.0	505.0	1028.0
Instar IV	850.0	1178.0	515.0	265.0	1329.0	738.0	450.0	677.0
Cocoon ( <i>N</i> )	814.8	492.0	270.3	108.5	89.3	972.0	584.0	547.5
Cocoon ( <i>I</i> )	236.7	284.1	35.4	28.4	18.07	390.2	384.9	461.9
Adult ( <i>A</i> )	60.4	36.9	10.0	6.6	0.0	194.2	142.2	175.5
Female (proportion of ♀ generation 2011 = <b>0.96</b> )	57.9	35.4	9.6	6.3	0.0	186.4	136.5	168.5
<b>Generation 2012</b>								
Egg estimated by female density x fecundity (38.1)	2207.9	1350.6	367.0	239.9	0.00	7102.1	5201.6	6419.8
Larval								
Instar I	59.5	326.8	225.5	86.6	51.4	427.5	865.8	296.5
Instar II	108.2	409.1	351.7	72.2	44.6	441.9	953.6	253.2
Instar III	93.8	300.9	245.3	30.7	74.4	434.7	864.6	246.8
Instar IV	34.3	296.5	257.9	9.0	44.6	568.2	827.3	190.5
Cocoon ( <i>N</i> )	15.6	35.3	16.4	9.1	9.6	69.8	52.9	33.2
Cocoon ( <i>I</i> )	NA	NA	NA	NA	NA	NA	NA	NA
*Adult ( <i>A</i> )	2.5	2.5	0	0	0	25.0	5.0	2.5

Fecundity of *Phistiphora erichsonii* (Hartig) = 38.1 was estimated by Higashiura (1988)

*N*: newly spun cocoons

*I*: unopened cocoons remaining after small mammal predation

*A*: adult emergence

\*: small mammal predation after sampling was not considered in the value of generation 2012



## **Appendix 2** Laboratory and analytical methods: needle chemical analysis

### *Carbon and nitrogen content*

Powdered needle (approximately 10 mg) from each sample was analyzed by using CHNS/O Analyzer (Perkin-Elmer PE2400 Series II).

### *Water-soluble sugars*

Powdered needle (approximately 20 mg) was suspended in micro centrifuge tube volume 1.5 ml by adding 1 ml of distilled water and mixed well on a vortex mixer. The sample was heated in heat block for 90 min at 50 °C, and then centrifuged 2000 rpm for 15 min. Phenol-sulfuric acid reagent was assayed to estimate water-soluble sugar using a method developed by DuBois et al. (1956) and modified by Chow and Landhäusser(2004). The supernatants of 0.04 ml were transferred to test tube and mixed with distilled water 0.36 ml. Then, 0.4 ml of 5% phenol solutions (W/V) were added, followed by 2 ml of 95% sulfuric acid. The mixture was gently mixed and incubated at room temperature. After 30 min, the absorbance was measured using spectrophotometer at 490 nm (UV-240A, Shimadzu). The standard curve was created with known concentrations of glucose.

### *Polyphenolic content*

Approximately 20 mg powdered needle were weighed into a test tube. Five ml of 50% methanol solutions (v/v) were added and mixed well on a vortex mixer. The test tube was then placed in an ultrasonic cleaner for 1 hr at 40 °C. The foliage particles were filtered and the filtrated solution was partitioned for total phenolics and condensed tannin analysis. Folin-Ciocalteu method used to determine total phenolics using a method developed by Lowman and Box (1983). Exactly 100 µl of solutions were placed in test tube and mixed with 2.15 ml purified water. Then, 250 µl of the Folin-Ciocalteu's phenol reagent solutions were added, followed by 2.5 ml of 20% sodium carbonate solutions (w/v). After mixing, the tube was incubated at room temperature for 20 min. The absorbance was measured at 700 nm with (UV-200A, Shimadzu). Total phenolics were expressed as tannic acid equivalents by comparison with calibration curves prepared from standard solutions. Condensed tannins were analyzed by a modification of the developed method by Cork and Krockenberger (1991) and Schofield *et al.* (2001). A 1 ml of solution was added to 4 ml of 5%

butanol-HCl solutions (v/v) in a screw cap tube. After mixing, the tube was placed in a thermo-regulated bath for 2 hours at 95 °C. The tubes were then cooled to room temperature and absorbance at 550 nm was measured. Cyanidin was used as standard for quantifying condensed tannins.

**Appendix 3** Defoliation intensity by *Pristiphora erichsonii* (Hartig), chemical and physical properties of needles, and length and the numbers of current-year long shoots on sample larch trees in seven study sites in the University of Tokyo Hokkaido Forest

Site	Sample tree No.	Group	Defoliation intensity (%)				Phenolics (%)			Tannins (%)			Sugars (%)			Carbon (%)			Nitrogen (%)			CN Ratio			Needle length (cm)			Needle weight (mg)			Shoot length (cm)				
			2009	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2009	2010	2011	2012	
1	2	1	0	100	100	Dead	7.85 (0.35)	16.2 (0.27)		2.81 (0.09)	6.05 (0.17)		16.0 (0.54)	16.1 (0.08)		48.2	47.5		2.35	1.58		20.5	30.1		2.79 (0.03)	2.45 (0.03)		1.640 (0.08)	1.670 (0.07)		6.1 (0.23)	6.1 (0.23)	5.3 (0.36)		
	29	1	10	100	90	10	6.22 (0.23)	12.0 (0.13)	15.4 (0.35)	2.20 (0.07)	4.04 (0.22)	6.87 (0.06)	14.8 (0.72)	13.9 (0.34)	18.7 (0.97)	47.2	46.6	48.1	1.98	1.59	1.12	23.9	29.3	43.0	2.33 (0.05)	2.11 (0.04)	1.81 (0.03)	1.373 (0.04)	1.527 (0.07)	1.227 (0.04)	9.3 (0.58)	11.2 (2.90)	7.0 (1.15)	7.1 (1.63)	
	27	1	0	60	80	0			13.26 (0.62)			3.92 (0.07)			18.90 (0.59)			48.5			1.43			33.9			1.72 (0.02)		0.927 (0.05)		14.7 (1.46)	8.7 (0.06)	6.6 (0.50)		
2	68	1	0	80	90	10	7.63 (0.14)	14.3 (0.21)	13.3 (0.57)	2.29 (0.07)	3.66 (0.15)	4.57 (0.08)	14.2 (0.72)	15.8 (0.56)	18.8 (0.61)	46.9	47.8	48.2	2.30	1.87	1.39	20.4	25.6	34.7	2.31 (0.02)	1.90 (0.02)	1.28 (0.02)	1.287 (0.03)	1.387 (0.05)	0.983 (0.06)	6.6 (0.29)	7.8 (0.99)	4.9 (0.78)	4.5 (0.31)	
	114	1	0	60	70	10	6.77 (0.11)	14.1 (0.24)	13.7 (0.48)	1.60 (0.12)	3.89 (0.15)	4.73 (0.15)	12.2 (1.58)	12.5 (0.21)	17.8 (0.78)	47.2	47.1	48.0	2.14	1.81	1.35	22.1	26.0	35.6	2.39 (0.05)	1.98 (0.03)	2.05 (0.02)	1.750 (0.07)	1.467 (0.05)	1.580 (0.07)	8.2 (0.21)	9.5 (2.03)	6.9 (1.59)	7.9 (0.66)	
	360	1	10	100	100	60	11.1 (0.31)	16.6 (0.24)	16.9 (0.41)	2.78 (0.10)	4.67 (0.14)	5.79 (0.07)	14.5 (1.91)	16.4 (0.93)	18.7 (0.79)	47.5	48.0	48.7	1.90	1.57	1.33	25.0	30.6	36.6	2.38 (0.02)	2.12 (0.02)	1.37 (0.02)	1.830 (0.42)	1.187 (0.27)	0.820 (0.04)	8.0 (0.49)	5.7 (0.04)	5.3 (0.42)	4.4 (0.73)	
	345	1	20	90	90	30	7.73 (0.19)	12.9 (0.35)	9.01 (0.65)	1.48 (0.12)	3.06 (0.23)	2.57 (0.05)	11.4 (1.44)	11.2 (0.47)	14.0 (1.23)	46.6	47.2	47.9	1.82	1.68	1.29	25.6	28.1	37.1	1.93 (0.04)	1.88 (0.03)	1.97 (0.02)	0.957 (0.05)	1.467 (0.05)	1.260 (0.06)	8.5 (0.96)	7.7 (0.92)	7.1 (0.84)	6.4 (0.16)	
	480	1	0	70	70	10	6.03 (0.22)	7.5 (0.25)	6.87 (0.67)	1.82 (0.09)	2.80 (0.16)	2.80 (0.08)	10.0 (0.00)	7.8 (0.00)	11.0 (0.32)	45.9	46.5	46.9	2.03	2.06	1.44	22.6	22.5	32.6	2.31 (0.03)	1.87 (0.02)	1.63 (0.02)	1.387 (0.04)	1.277 (0.05)	1.157 (0.01)	9.8 (0.87)	7.6 (0.09)	6.5 (0.32)	5.8 (0.05)	
	476	1	10	50	70	20	15.70 (0.40)	22.3 (0.71)	15.65 (0.58)	3.21 (0.19)	5.27 (0.14)	3.63 (0.07)	16.7 (0.01)	20.3 (0.01)	16.1 (0.81)	47.9	47.7	47.6	1.68	1.56	1.4	28.5	30.6	34.0	2.06 (0.04)	2.02 (0.04)	1.86 (0.02)	1.293 (0.06)	1.977 (0.06)	0.913 (0.04)	9.4 (1.15)	10.2 (1.21)	8.0 (1.81)	8.5 (0.32)	
5	207	1	0	70	80	10	10.5 (0.15)	10.7 (0.02)	10.2 (0.54)	3.30 (0.10)	3.20 (0.04)	3.60 (0.15)	11.1 (0.76)	14.6 (0.42)	16.3 (0.87)	47.5	46.4	47.8	2.48	1.73	1.61	19.1	26.9	29.7	2.54 (0.03)	2.52 (0.04)	2.63 (0.03)	1.227 (0.04)	1.363 (0.10)	1.703 (0.10)	9.5 (0.18)	8.0 (0.50)	6.1 (0.55)	9.4 (1.37)	
	209	1	20	100	100	10	9.71 (0.16)		12.0 (0.45)	3.13 (0.15)		4.56 (0.09)	13.4 (0.76)		17.8 (0.86)	47.2		47.5	2.29		1.48	20.6		32.1	1.72 (0.03)		1.51 (0.02)	0.423 (0.02)	0.857 (0.04)		7.9 (1.09)	3.5 (3.53)	3.5 (0.78)		
	165	1	0	70	90	10		12.0 (0.01)	12.5 (0.56)		3.84 (0.01)	5.06 (0.08)		16.9 (0.08)	17.2 (1.73)		47.2	48.7		1.61	1.25		29.3	38.9			1.83 (0.03)	1.55 (0.02)		1.220 (0.04)	1.080 (0.05)	9.6 (1.23)	9.2 (0.39)	5.2 (0.77)	3.9 (0.65)
6	304	1	0	100	80	60	10.8 (0.29)	13.5 (0.32)	13.3 (0.33)	2.74 (0.17)	3.43 (0.19)	4.03 (0.16)	15.9 (0.77)	16.0 (0.13)	18.0 (0.92)	45.8	47.4	47.8	1.61	1.63	1.31	28.5	29.1	36.5	2.47 (0.03)	2.29 (0.03)	1.48 (0.02)	1.813 (0.07)	1.913 (0.08)	1.207 (0.05)	9.7 (1.16)	8.6 (0.45)	6.7 (0.24)	6.6 (0.08)	
	303	2	100	100	90	50	13.8 (0.28)	19.3 (0.21)	17.3 (0.19)	3.62 (0.16)	6.15 (0.06)	6.02 (0.19)	16.1 (0.50)	21.0 (0.81)	19.6 (0.67)	47.1	47.9	48.2	1.75	1.25	1.25	26.9	38.3	38.6	2.01 (0.03)	1.52 (0.02)	1.05 (0.01)	1.157 (0.04)	1.397 (0.06)	0.707 (0.03)	11.4 (0.63)	7.6 (0.67)	5.2 (0.48)	5.6 (0.22)	
	382	2	80	90	90	80	11.6 (0.17)		13.6 (0.56)	3.41 (0.23)		4.12 (0.05)	11.7 (0.55)		19.8 (0.78)	46.6		47.2	1.44		1.41	32.3		33.4	1.73 (0.02)		1.64 (0.02)	1.147 (0.04)		1.093 (0.06)		8.0 (0.33)	6.6 (0.36)	6.6 (0.22)	
	439	2	70	70	10	20	12.4 (0.22)		14.4 (0.55)	2.58 (0.06)		3.41 (0.07)	13.9 (0.40)		18.7 (0.82)	47.4		48.6	1.59		1.44	29.8		33.8	2.17 (0.04)		1.82 (0.02)	2.073 (0.06)		1.487 (0.07)		9.1 (0.66)	7.2 (0.86)	6.3 (0.49)	
	440	2	90	90	80	90	12.6 (0.19)	13.4 (0.16)	13.0 (0.48)	3.57 (0.18)	4.06 (0.24)	4.45 (0.08)	17.0 (0.17)	13.7 (0.88)	19.5 (0.77)	47.3	48.1	48.2	1.59	1.62	1.31	29.7	29.7	36.8	2.30 (0.04)	2.34 (0.03)	2.10 (0.03)	1.340 (0.05)	1.540 (0.04)	1.283 (0.05)	11.2 (1.71)	10.1 (0.74)	7.0 (0.31)	7.4 (0.10)	
	442	2	100	100	100	90	9.95 (0.06)	14.1 (0.16)	13.2 (0.46)	2.41 (0.08)	3.58 (0.33)	3.93 (0.10)	12.3 (0.31)	14.4 (0.52)	17.1 (0.28)	46.4	48.0	48.6	1.46	1.60	1.27	31.8	30.0	38.2	1.88 (0.04)	1.50 (0.02)	1.25 (0.02)	1.193 (0.05)	1.223 (0.05)	0.883 (0.05)	10.6 (0.95)	9.3 (1.75)	5.7 (0.49)	5.9 (0.14)	
8	74	1	0	100	90	10	11.0 (0.11)	12.0 (0.26)	11.7 (0.53)	2.10 (0.12)	2.57 (0.17)	3.13 (0.06)	10.3 (0.75)	13.7 (0.24)	16.1 (0.31)	47.2	47.3	48.0	1.72	1.66	1.48	27.4	28.5	32.4	2.27 (0.02)	2.01 (0.02)	1.14 (0.01)	1.703 (0.04)	1.517 (0.05)	0.910 (0.03)	7.2 (0.66)	7.6 (0.38)	7.7 (0.09)	4.0 (0.16)	
	75	1	20	80	70	10	11.20 (0.16)	13.1 (0.61)	12.2 (0.44)	2.06 (0.15)	2.94 (0.08)	3.37 (0.07)	11.3 (0.45)	16.3 (0.82)	17.6 (0.48)	47.4	47.1	48.5	2.33	1.50	1.07	20.3	31.4	45.3	2.14 (0.04)	1.82 (0.03)	1.46 (0.02)	1.940 (0.07)	2.113 (0.09)	1.480 (0.06)	14.2 (1.04)	10.0 (0.92)	7.6 (0.08)	6.2 (0.15)	
	mean (SE)																																		