Doctoral dissertation

Time-scale dependency of plant biomass- and trait-mediated indirect effects of large herbivores on phytophagous insects

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March 2012

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Abstract

Species interact with each other both directly and indirectly, and indirect effects have similar or even stronger impacts on population dynamics and community structure than direct effect. Indirect effects were divided into two main processes based on the mode of transmitter response; density-mediated indirect effects (DMIEs) and trait-mediated indirect effects (TMIEs). To predict the indirect interactions caused by multiple processes, researchers need to detect the factor affecting the relative strength of these processes. Because there are differences in response speeds between trait changes via phenotypic plasticity at the individual level and demographic changes at the population level, the relative importance of these effects is expected to show time-scale dependency. However, no empirical study provided evidence supporting time-scale dependency of relative strength of DMIEs and TMIEs.

The purpose of this study is to demonstrate the time-scale dependency in density- and trait-mediated indirect effects in "large herbivore-plant-phytophagous insects" systems, in which plant quantity- and quality-mediated interactions were widely known. First, I explore general patterns in the direction of indirect effects of large herbivores on insect densities. Second, I examined indirect interactions between sika deer (*Cervus nippon*)-woody pipevine (*Aristolochia kaempferi*)-swallowtail butterfly (*Byasa alcinous*) in the Boso Peninsula, and estimated the time-scale dependency of plant quantity- and quality-mediated effects.

Chapter 2 explores general patterns in the directions of indirect effects of large herbivores on insect densities. Large herbivores affect insect abundance both positively and negatively, or did not show significant impacts. The directions of total effects are determined by the relative strength of the positive and negative effects, which is assumed to associate with plant qualitative and quantitative changes induced by browsing. I focused on three factors associated with the appearance of density-mediated indirect effects, i.e., plant life-form, measures of insect density, and time-scale of study. As a result, differences in these factors affected the direction of indirect effects. Studies mediated by forb or grass exhibited negative effects on insect abundance in long-term studies, whereas studies mediated by trees tended to show positive effects on attack rates by insects in short-term studies. Consideration of the above three factors may help predict the occurrence of negative effects in nature.

Chapter 3 investigated the processes of trait-mediated indirect effects in the "sika deer – woody pipevine – swallowtail butterfly" system. First, to assess how *A. kaempferi* responds to deer browsing, I conducted field experiments using deer exclosures. Outside the exclosures, deer browsing stimulated the regrowth of *A. kaempferi*. Simulated browsing also induced compensatory growth of *A. kaempferi* and improved leaf nutritional and physical qualities for insects. Second, to examine the young leaf utilization by *B. alcinous* in response to increasing deer densities, I conducted field survey on the availability and utilization patterns of young leaves across deer-density gradient. Young leaf availability increased with

deer density, and young leaf utilization by butterflies also increased. Third, to examine the effects of plant trait-change on larval and pupal performance of *B. alcinous*, I conducted laboratory experiments raring larvae at different food-quality treatments. *Byasa alcinous* larvae fed by good quality leaves increased their survival rate, growth rate, and decreased the incidence of pupal diapause. Consequently, the combination between plastic response of *A. kaempferi* to deer browsing and plastic response of *B. alcinous* to food quality may lead to positive trait-mediated effects.

Chapter 4 evaluated the effect of deer on the density of *B. alcinous* mediated by the changes in quantity and quality of *A. kaempferi*. The density of sika deer population in the Boso Peninsula was estimated for 15 years, and areas with different browsing histories are available. I conducted the field survey for *A. kaempferi*, and estimated the carry-over rate of responses of plant quantity and quality by the relationship between plant response and temporal trends in deer density. Leaf density of *A. kaempferi* exhibited high carry-over rate of the effect of deer browsing and cumulatively decreased in response to successive deer herbivory, while percentage of young leaves increased with current deer density. The high density of *B. alcinous* were observed in areas with high leaf density and high percentage of young leaves. Total estimated indirect effects showed a positive trend in short-term due to relatively strong quality-mediated effect, but they showed no specific trends in long-term because quality- and quantity- mediated effects canceled each other.

According to these results, I found that the indirect effects of large

herbivores on insects mediated by host plants exhibit a time-scale dependency. This study provides the first empirical evidence supporting the time-scale dependency of the relative importance of density- and trait-mediated effects. In large herbivore-plant insects system, the transmitter plants show slow and cumulative responses, which may cause time-scale dependency. In trophic cascade studies, transmitter species often have a short generational time and sometimes the recruitment to the local population in the following year is limited by large spatial-scale processes. In such cases, cumulative responses over years are unlikely to appear in small spatial scale experiments. In nature, inducer populations can show both fast- and slow-time scale dynamics, which may cause the rapid trait changes and the cumulative density changes in transmitter. To understand indirect interactions under the transient conditions, researchers should be in mind the existence of time-scale dependency.

Chapter 1: General introduction

In ecological communities, species interact with each other both directly and indirectly through predation, competition, and mutualism. Each species responds at different spatio-temporal scales, and the strength of responses may occasionally show context-dependencies. To understand population dynamics and species interactions within a community, researchers need to clarify what types of responses underlie the indirect interactions.

In the 1960's, classical studies showed the importance of indirect effects on community structures in rocky intertidal systems. In Paine's experimental study (Paine, 1966), the removal of sea stars, known as a top predator, resulted in the reduction of species richness of sessile organisms. This phenomenon, known as keystone predation, was caused by the indirect effect of predator removal on prey organisms mediated by a competitive dominance of the mussel. As this example shows, the indirect effect of inducer species on responder species via density changes in transmitter species is called "density-mediated indirect effect" (DMIE; Abrams et al., 1996). After the 1970's, a large number of theoretical and experimental studies focusing on this type of indirect interactions were conducted. Meta-analyses showed that DMIEs had similar or even stronger impacts on communities than direct effects (Menge, 1995; 1997). Furthermore, indirect effects mediated by changes in transmitter traits were also demonstrated (trait-mediated indirect effect; TMIE; Abrams et al., 1996), whereby the

effects of inducer were transmitted by traits of transmitter, including anti-predator behavior (e.g., Huang & Sih, 1990), induced defensive morphology (e.g., Raimondi et al., 2000), and chemical compounds in plant tissues (e.g., Martinsen et al., 1998). Although studies on TMIEs were less common than on DMIEs, TMIEs are widely known in both terrestrial and aquatic ecosystems (Werner & Peacor, 2003). For example, TMIEs have been demonstrated in terrestrial plant-herbivorous insect systems in which DMIEs had been considered to have relatively little importance for community structures (Ohgushi, 2005; Kaplan & Denno, 2007). Many studies demonstrated the existence of trait-mediated interactions, but there remain many problems to generalize its importance: How is the relative importance of TMIEs relative to DMIEs? Under what conditions do TMIEs have stronger impacts? A general framework is needed for understand the indirect interactions caused by multiple processes.

Most of the earlier studies were designed to demonstrate the existence of trait-mediated interactions, but recent researchers paid more attention to quantify the relative strength of density- and trait-mediated indirect effects (Okuyama & Bolker, 2007). A meta-analysis demonstrated that TMIEs have relatively stronger effects than DMIEs (Preisser et al., 2005), but the relative importance of these effects is context-dependent. In interactions between three vertical trophic chains (top-down trophic cascade), consumption/defense trade-off in prey behavior is a key determinant of the strength of TMIEs. Because of higher predation risks or lower consumption benefits, stronger TMIEs might have appeared under conditions with higher

predation risk (Trussell et al., 2006) or lower resource availability (Wojdak & Luttbeg, 2005). In addition, time-scale of experiments was suggested as a factor affecting the relative importance of DMIEs and TMIEs (Werner & Peacor, 2003). Time-scale dependency is arisen because of the differences in response speeds between trait changes via phenotypic plasticity at the individual level and demographic changes at the population level. Time-scale dependency was expected to occur in apparent competition (or apparent mutualism) (Holt & Kotler, 1987), which does not assume the trade-off of density- and trait-changes. However, as far as I know, no empirical studies provided evidence supporting time-scale dependency of relative importance of density- and trait-mediated indirect effects. This is probably because of the difficulties in conducting long-term experiments that could detect multi-generational cumulative responses. The changes in density of inducer species affect transmitter species in a short time scale as pulse perturbation, while these could affect the equilibrium of the system as a whole in a long time scale as press perturbation (Bender et al., 1984). Inducer populations show both fast and slow time scale dynamics in nature. Thus, considering time-scale dependency may be the key to understand indirect interactions under fluctuating environments, and researchers should pay more attention to how densities and traits of transmitter respond across time scales.

The purpose of this study is to demonstrate the time-scale dependency in density- and trait-mediated indirect effects in a "large herbivore-plant- phytophagous insect" system. Browsing or grazing by large herbivores reduces plant biomass and modifies plant traits including

physical architectures, secondary compounds, and nutritional contents, which are likely to cause indirect effects on phytophagous insects (Nykänen & Kolicheva, 2004; Suominen & Danell, 2006; Gómez & González-Megías, 2007a). Indirect interactions in this system are asymmetric; indirect effects from large herbivores to insects are dominant because of the large difference in the body size (Gómez & González-Megías, 2007a). Large herbivores are one of the primary disturbance agents in terrestrial ecosystems and have huge direct and indirect impacts on ecosystems (Rooney & Waller, 2003; Côte et al., 2004). Understanding the processes and consequences of indirect effects by large herbivores on insects will contribute to predict ecological impacts of overabundant large-herbivores.

This thesis consists of the following sections. Chapter 2 explores general patterns in the directions of indirect effects of large herbivores on insect densities. I reviewed studies on indirect effects in the system consisting of large herbivore-plant-phytophagous insects. Negative- and positive-processes are assumed to underlie the indirect effects on insects, and the total effects are assumed to depend on the relative strength of these processes. The presence or strength of the effects mediated by plant biomass reduction may depend on the characteristics of transmitter plants and/or experimental design. I conducted meta-analyses to test whether the direction of indirect effects on phytophagous insects are related to life-form of transmitter plants, types of density measures, and time-scale of the experiments. Chapters 3 and 4 examined indirect interactions between sika deer (*Cervus nippon*)-woody vine (*Aristolochia kaempferi*)-swallowtail

butterfly (*Byasa alcinous*) in the Boso Peninsula, Japan, where spatial trends in deer density had been estimated in the past 15 years. This could provide the opportunity to estimate the strength and duration of the deer effect by the comparisons of plant and insect responses across a deer density gradient. Because *B. alcinous* uses only the genus *Aristolochia* as a host plant, changes in biomass and/or traits of *A. kaempferi* are likely to influence resource quantity and/or quality for *B. alcinous*. Chapter 3 investigated the processes of trait-mediated indirect effects in this system. First, to assess how *A. kaempferi* responds to deer browsing, I conducted field experiments using deer exclosures. Second, to examine the young leaf utilization by *B. alcinous* in response to increasing deer densities, I conducted field survey on the availability and utilization patterns of young leaves across deer-density gradient. Third, to examine the effects of plant trait-change on larval and pupal performance of *B. alcinous*, I conducted laboratory experiments raring larvae at different food-quality treatments. Chapter 4 evaluated the effect of deer on the density of *B. alcinous* mediated by the changes in quantity and quality of *A. kaempferi*. I hypothesize that the density of *A. kaempferi* responds to cumulative browsing history while its quality responds to current browsing pressure. I estimated the carry-over rate of responses of plant quantity and quality by the relationship between plant response and temporal trends in deer density. Chapter 5 discusses the generality of time-scale dependency of indirect effects by the comparison with trophic cascade systems.

Chapter 2: General patterns of indirect effects in "large herbivore-terrestrial plant-phytophagous insect" systems

2.1. Introduction

Phytophagous insects are expected to be strongly impacted by large herbivores because they are largely dependent on plants for food and habitat. Many previous studies reported the effects of livestock and wild ungulates on the density and species richness of phytophagous insects (Suominen & Danell, 2006). Large herbivores affect insect abundance both positively (e.g., Elligsen et al., 1997; Seymour & Dean, 1999), and negatively (e.g., Kruess & Tscharntke, 2002; Debano, 2006), or did not show significant impacts (Mysterud et al., 2005). Understanding the background processes causing positive and negative effects may help predict such context dependencies.

For insects using plants palatable to large herbivores, reductions in biomass of host plants can result in depletion of food resource or habitat. Plant quality also changes after herbivory through compensatory growth or induced resistance, which are associated with plant tolerance and resistance strategies, respectively (Bryant et al., 1991). Both positive and negative effects were reported, but plant qualitative changes induced by mammalian browsing tend to affect insect performance positively in comparison with the changes induced by insect defoliation (Nykänen & Koricheva, 2004). The directions of total effects are determined by the relative strength of the positive and negative effects. In other words, negative effects are expected to be observed under conditions when density-mediated process is stronger.

Detecting factors affecting the relative strength of quantity- and quality-mediated processes may help predict the direction of these inconsistent effects.

Here, I focused on three factors associated with the appearance of density-mediated indirect effects. First, life-form of transmitter plants may affect susceptibility to quantity and quality mediated effects. For example, mammalian grazing is likely to reduce aboveground biomass of graminoids (Diaz et al., 2007), while it may stimulate compensatory growth for tall trees having nitrogen stock in their stem (Millard et al., 2001). Studies on plant-mediated effects of large herbivores on insects were conducted in various ecosystems including pasture, savanna, and forests consisting of plants with different life forms. I expected that indirect effects mediated by tall trees exhibit negative effects less frequently than those mediated by shrubs or forbs because trees may suffer browsing only small part of the plant body. Second, the appearance of quantity-mediated effect may depend on the measures of insect density. There have been two different measures that were used for estimating the effects on insect density, i. e., the density per unit area (hereafter 'abundance') and the density per unit plant part (hereafter 'attack rate') including individual plant, plant shoot, and unit number of leaves. Because 'abundance' of insects is affected by the decrease in plant density per unit area as well as the decrease in attack rate, studies using abundance as a measure of density are likely to have frequent appearance of negative indirect effects. Third, the time-scale of the studies may affect the occurrence of cumulative quantity-mediated effect. Biomass

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recovery of slowly growing plants is unlikely to be detected in two- or three-year herbivore exclusion (e.g., Olofsson, 2006). Even after 40-years deer culling, vegetation was not completely recovered (Tanentzap et al., 2009). On the contrary, compensatory growth after browsing may respond to the current year browsing, and usually unrelated to the past browsing history (Strauss, 1991). The time scale of earlier studies ranged from one year to several decades. I expected that studies conducted in longer time scale may tend to exhibit negative effects on insects.

In this chapter, I explore the general patterns of the indirect effects of large herbivores on phytophagous insects through host plants. I paid special attentions to (1) plant life-form, (2) measures of insect density, and (3) time-scale of study. Specifically, the following questions were addressed: (1) Do forbs and shrubs have a tendency to transmit negative effect more than trees? (2) Do studies using abundance as a response variable have a tendency to show negative effects more than attack rate? (3) Do longer-term studies have a tendency to show negative effects?

2.2. Materials and Methods

I analyzed 177 observations from 44 studies that estimated indirect effects of large herbivores on density of phytophagous insects (Appendix). Studies estimating the effects on species, genus, family, and order level densities were included in the analysis. Hemipteran, Orthopteran, and Lepidopteran insects were regarded as phytophagopus insects.

Life-forms of transmitter plants were classified into three types:

[forb/grass], [shrub], and [tree]. Studies that did not explicitly identify the type of transmitter plants were classified as [forb/grass] when they were conducted in grasslands or pastures. The [shrub] category also included tree seedlings, saplings, and juveniles. A total of 159 observations were categorized into either one of the three, but 18 cannot.

Measures of density of insects were classified into two types: [abundance] and [attack rate]. 108 studies estimating the effects on density per unit area were categorized as [abundance] studies, and 69 studies estimating the effects on density per unit (parts of) plant were categorized as [attack rate].

Years after the establishment of exclosures or the beginning of livestock management were not always accurately identified (e.g., described as "at least ~ years"). Because about half the number of studies were conducted in time scale shorter than 5 years, the time scale of the studies was divided into two categories; [short] (< 5 years) and [long] ($\geq$ 5years). Number of [short] and [long] studies were 60 and 65, respectively, and 52 could not be classified.

Because the above studies included effects from various species and various densities of herbivores, estimating the combined effect size seemed to be inappropriate, and instead the direction of indirect effect was examined. Observations including both positive and negative effects in a given study were categorized as neutral effect, and were omitted for further statistical tests. Not significant effects were also classified into positive or negative observations. The signs of observations were tested separately for each of the

following factor; (1) plant life-form, (2) measures of density, and (3) time-scale. Whether the ratio of positive and negative effects differ among levels within each factor was tested by χ² test or Fisher's test. The direction of the indirect effects for each level of the factor was tested by sign test, and significance levels were corrected by sequential Bonferroni method. As there was an association between factors, I tallied the number of observations in each combination of factors, and sign test was performed for each combination except when the number of observations was too small (less than 5).

2.3. Results

There were 92 negative effects and 58 positive effects, and 27 were uncategorized. Negative effects were more common than positive effects but the difference was not significantly ($P = 0.003$). Among 159 observations for which transmitter plant types were identified, 87, 46, and 26 observations belonged to [forb/grass], [shrub], and [tree] category, respectively (Table 2.1). The directions of indirect effects depended on transmitter type ($P < 0.001$), i.e., trees tended to transmit positive effects, whereas forbs/grasses tended to transmit negative effects. There were 108 observations for which the effects were estimated by [abundance] change, and 69 observations estimated by [attack rate]. The directions of indirect effects depended on measures of density ($P < 0.001$), i.e., abundance per area was negatively affected by large herbivores, whereas attack rate per plant was positively affected. There were 60 and 65 observations for which the experiments were conducted in [short]

and [long] term, respectively. The directions of indirect effects depended on transmitter type ($P = 0.008$), i.e., long-term studies tended to show negative effects, but short-term studies did not show a specific trend.

There was a strong association between plant type and measures of density (Table 2.2). Almost all the studies targeting on [forb/grass] estimated [abundance], while studies on [shrub] or [tree] species estimated [attack rate]. Studies mediated by forb/grass exhibited negative effects on insect abundance, and a significant effect was detected in long-term studies (Table 2.2a; $P < 0.001$). For attack rate, shrub mediated effects did not show any particular trends. Studies mediated by trees tended to show positive effects on attack rates by insects, and a significant effect was detected in short-term studies (Table 2.2b; $P = 0.002$).

2.4. Discussion

Studies on the indirect effects of large herbivores on phytophagous insects consisted of various transmitter plant types, response variables, and time-scales. Differences in these factors affected the direction of indirect effects. Transmitter plant types were associated with response variable, i.e., in grasslands, indirect effects on insects were estimated mostly by the changes in their abundance per area, and they exhibited negative trends. In contrast, indirect effects mediated by trees or shrubs were estimated by the changes in attack rate on plant individual or module. Even if density of insects per plant was not affected, population density of insects decreased when plants were reduced by herbivore grazing. I suggest that to estimate

the indirect effects of large herbivores on insect populations, abundance per area, not per plant, should be used as response variables. However, abundance-based estimation of indirect effects is not always sufficiently suitable. For example, when large herbivores affect insect metapopulations in large-scale (e.g., Schtickzelle et al., 2007), a comparison between grazed patches vs. non-grazed patches is inappropriate to estimate the population-level impacts. A comparison of networks consisting of grazed-patch dominated vs. non-grazed-patch dominated will be a better assessment of population level effects. Depending on the purpose of studies, researchers need to recognize what level of response (e.g., behavioral response or numerical response) was examined in each study.

I also found that plant life-form influenced the direction of indirect effects, i.e., tree-mediated effects tended to result in positive consequences more often than shrubs and grasses. This could be explained by several non-exclusive reasons. First, almost all the indirect effects were estimated by attack rate level in trees, which probably led to underestimation of negative effects mediated by plant biomass reduction. Because plant qualitative changes induced by browsing often improve insect performance (Nykänen & Koricheva, 2004), positive effects may have prevailed in total indirect effect. Another possible reason is due to higher browsing-tolerance of tree species. Shrubs and herbs have a high risk of being browsed of their whole aboveground tissues, but trees are unlikely to be browsed of their whole growing tissues. A study on the indirect effect of cattle on galling aphids mediated by two *Pistacia* species (Martinez & Wool, 2003) also supported

this pattern. In the pruned treatment, *P. atlantica*, a tall-tree species, sufficiently compensated its tissue loss and was attacked by more aphids, whereas *P. palaestina*, a shrub species, reduced its shoot length and number of leaves and was attacked less frequently than in the unpruned treatment. In addition, insect characters may also affect this pattern. Insects utilizing trees often inhabit both understory and tree crown. Because browsing by large herbivores on understory influences only insects inhabiting understory, population level effects are likely to be minimal.

Long term studies had a tendency to exhibit negative effects, and there were no significant positive trends except for short-term studies mediated by trees. The finding that longer experiments tend to show more negative effects was supported by a 7-year experiment (Gómez & González-Megías, 2007b), which showed that the increase in abundance of seed predators appeared 6-7 years after ungulate exclusion but not 2-3 years after. It appears that short-term experiments may underestimate the occurrence and strength of plant density-mediated indirect effects which prevail in long-term experiments.

Two other factors may also influence the direction or strength of the indirect effects, i.e., the nature of large herbivores and habitat productivity. Exotic herbivores are expected to have relatively strong negative impacts on insects inhabiting native plants having lower resistance or tolerance to exotic herbivores (Bailey & Schweitzer, 2010). Also, plants can compensate for biomass losses to herbivory in productive environments, but they are unable to do so in environment with low productivity (Persson et al., 2007).

Therefore, quantity-mediated indirect effects are likely to be dampened in highly productive habitats (Pringle et al. 2007). Examining the effects on species interactions by global environmental changes such as biological invasion, climate change, and nitrogen deposition is one of the important ecological issues to be addressed in the future (Tylianakis et al., 2008).

I propose several prospects for future research. First, researchers should assess indirect effects at the population level of insects. For the assessment of population viability or conservation of endangered species, estimation based on the attack rate per plant may underestimate negative effects. In particular, for insects using shrub or tree species, most studies examined their density per plant individual or module. Indeed, for insects having high mobility and broad population scales, large-scale experiments are difficult to conduct at the population level. In such situations, comparative studies using gradients of large herbivore densities are promising. Second, plant quantity- and quality-mediated effects should be separately estimated. These two processes have not always been separated explicitly. Although plant trait-mediated effects are widely known (Ohgushi, 2005), the relative strength of density- and trait-mediated effects has been largely unknown. The relative strength of density- and trait-mediated effects was well studied in trophic cascade systems (e.g., Preisser et al., 2005), but rarely addressed in other systems. Third, more studies should be conducted in long time scales. Extrapolating the effects estimated in short-term studies may underestimate the negative effects actually found in longer-term scales. Although there have been a few studies on plant trait-mediated indirect

effects over 5-y scales (Miyamoto, 2006), long-term studies may be important for generalizing the relative strength of density- and trait-mediated indirect effects.

There have been narrative reviews examining the effects of large herbivores on insects (e.g., Stewert, 2001; Suominen & Danell, 2006; Gómez & González-Megías, 2007a). However, under which conditions do negative and positive processes prevail have not been analyzed. Here, we found that transmitter plant types, measures of insect densities, and time-scale of study influence the direction of indirect effects, which might result in the context dependency of the strength or occurrence of plant quantity-mediated process. Although underlying processes were often unidentified in indirect interaction systems, consideration of the above three factors may help predict the occurrence of negative effects in nature.

Table 2.1.

The relationship between the sign of indirect effects and (1) plant type, (2) measures of densities, and (3) time scale. Numbers indicate the incidence of observations in each category. *P* values are from sign test. Asterisks indicate significant relationships after sequential Bonferroni correction.

	sign of indirect effects				
	negative	neutral	positive	*P*	
total observations	92	27	58	0.003	
(1) plant type					
forb/grass	62	8	17	<0.001	*
shrub	19	9	18	0.5	
tree	3	5	18	<0.001	*
(2) measures of densities					
abundance	71	15	22	<0.001	*
attack rate	21	12	36	0.031	*
(3) time scale					
short	20	11	29	0.126	
long	36	12	17	0.006	*

Table 2.2.

The sign of indirect effects on (a) abundance and (b) attack rate of insects mediated by each plant type in short and long-term studies. Numbers indicate the incidence of observations in each category. P values are from sign test, and bold letters mean significant effects after sequential Bonferroni correction.

(a) effects on abundance

	time scale					
	short			long		
plant type	(-)	0	(+)	(-)	0	(+)
forb/grass	6	1	2	20	5	4
		$P = 0.063$			**$P < 0.001$**	
shrub	0	0	0	1	0	0
		No test			No test	
tree	0	0	0	1	0	0
		No test			No test	

(b) effects on attack rate

	time scale					
	short			long		
plant type	(-)	0	(+)	(-)	0	(+)
forb/grass	1	0	0	1	0	0
		No test			No test	
shrub	9	14	15	7	3	3
		$P = 0.154$			$P = 0.172$	
tree	1	3	12	1	2	6
		$P = 0.002$			$P = 0.063$	

Chapter 3: Trait-mediated indirect effects in the "sika deer – woody pipevine – swallowtail butterfly" system

3.1. Introduction

Browsing or grazing by large herbivores decreases plant density and modifies plant traits, which indirectly affects phytophagous insects. In general, reductions in host plant biomass affect abundance of phytophagous insects in a negative way, whereas plant qualitative changes influence insect density both positively (e.g., Martinsen et al. 1998; Bailey & Whitham, 2006) and negatively (e.g., Shimazaki & Miyashita, 2002). Many of the insect life-history traits including growth rate, fecundity, and survival rate are highly dependent on their host plant quality (Awmack & Leather, 2002). Furthermore, strength and direction of plant-mediated effects on phytophagous insects depend on life-historical stages of insects (Tabuchi et al., 2010). To understand the mechanisms of trait-mediated indirect effects, we need to investigate how plant quality affects life-historical stage of insects.

Leaf age is a major source of intraspecific variation in plant quality. Insects often prefer young leaves because of their high nitrogen content and low physical defense (Coley & Barone, 1996; Wait et al., 2002; Utsumi et al., 2009). In temperate areas young leaves are generally unavailable to insects except during flush periods of their host plants, but compensatory growth induced by biotic and abiotic disturbances supply young leaves aseasonally (Spiller & Agrawal, 2003; Nakamura et al., 2005; Utsumi et al., 2009). For

example, browsing by large herbivores (a biotic disturbance) induces plant regrowth and secondary shoots, which often supports high abundance and performance of phytophagous insects (e.g., Martinsen et al., 1998).

It has been often reported that there are differences in densities of insects and their eggs on browsed and unbrowsed plants (Martinsen et al., 1998; Olofsson & Strengbom, 2000; Bailey & Whitham, 2006). However, exclosure experiments conducted in an area with a given large-herbivore density could not predict "dose response" of insects to increasing large herbivore densities when insects show non-linear responses such as a threshold or unimodal reaction and/or saturation (Suominen & Danell, 2006). To resolve this problem, it is necessary to investigate the availability and utilization patterns of suitable plant resources by insects in response to the density of large herbivores.

In this chapter, I examine the effects of regrowth of a host plant (Aristolochiae: *Aristolochia kaempferi* Wild.) induced by sika deer (Cervidae: *Cervus nippon* Temmink) on a swallowtail butterfly (Papilionidae: *Byasa alcinous* Klug). *Aristolochia kaempferi* is the only host plant of *B. alcinous* in the study area. Leaves of this plant usually matured in summer, but young leaves are observed even in summer in habitats where farmers frequently mow (Takagi & Miyashita, 2008). Thus, it is considered that in areas with little biotic or abiotic disturbance, young leaves are rarely available in summer; however, if deer browsing induces regrowth of *A. kaempferi*, young leaves are expected to be available during the summer. If adult females of *B. alcinous* selectively oviposit on young leaves, utilization of young leaves

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should be higher than that expected from the proportion of young leaves. In addition, the utilization of young leaves by the butterfly is likely to reach a saturation level in areas with a high availability of young leaves. High utilization of young leaves is supposed to improve the performance of *B. alcinous*. Populations using *A. kaempferi* have a higher tendency to enter summer diapause, which is thought to be an adaptive strategy for escaping the period when young leaves are unavailable (Kato, 2001). As inductions of reproductive diapause by low food-quality has been reported in some herbivores (Hare, 1983; Ishihara & Ohgushi, 2006; Ito & Saito, 2006), the regrowth induced by deer browsing may lead to high larval performance and low pupal diapause of *B. alcinous*.

To explore the processes of trait-mediated indirect effects in "sika deer-*A. kaempferi*-swallowtail butterfly" system, I addressed the following three questions.

(1) How does deer browsing affect the leaf quality of *A. kaempferi*? To investigate the induction of compensatory growth and the improvement of nutritional or physical quality, an exclosure experiment and a simulated browsing experiment were conducted.

(2) How does the utilization of good quality leaves by *B. alcinous* change with increasing deer density? To examine the patterns of the frequency of young leaves of *A. kaempferi* and the frequency of young leaves oviposited by *B. alcinous*, a field survey across a deer-density gradient was conducted.

(3) How does the utilization of good-quality leaves influence larval or

pupal performance of *B. alcinous*? To examine the plant quality on larval survival, growth rate, and pupal diapause, the performances of *B. alcinous* fed on good- and poor-quality leaves were compared by a laboratory experiment.

3.2. Materials and Methods

3.2.1. Study system

Field experiments and surveys were conducted on the Boso Peninsula (15 × 30 km; 35.12-.26 °N, 139.95-140.23 °E; altitude 100-200 m; Figure 3.1), central Japan. The climate is warm temperate, with mean temperatures of 25 °C in midsummer and 4 °C in midwinter. Annual precipitation is 2000-2400 mm. The dominant vegetation is broad-leaved evergreen forest (e.g., *Castanopsis sieboldii* [Makino] Hatus and *Quercus* spp.) and coniferous plantations (*Cryptomeria japonica* D. Don and *Chamaecyparis obtusa* [Siebold & Zucc.] Endl.).

Sika deer were once restricted to a small area (40 km²) of the Boso peninsula as a result of overharvesting, but the population began increasing in the early 1970s and now extends over an area of 1000 km² (Asada, 2011). Deer density varies locally; 10-20 individuals / km² of deer have been established at the center of their distribution, and c.a. < 10 individuals / km² of deer inhabit in peripheral areas. The density of the entire distribution of sika deer has been estimated at a resolution of 1 km² (methods detailed in Suzuki et al., 2008) by determining the density of fecal pellets. The deer density for this study was estimated by pellet counts in 2007 (Chiba

Prefecture, 2008). This estimation was considered a reliable measure of actual local deer density, because fecal pellet density showed a high correlation ($r^2 = 0.731$, $n = 14$, $P < 0.001$; Chiba Prefecture, 1998) with local deer density estimated by a block counting method (Maruyama & Nakamura, 1983), and the spatial gradient of pellet densities of the Boso sika deer population has been fairly stable since 2000 (Miyashita et al., 2008).

Byasa alcinous is a swallowtail butterfly found in eastern Asia. The life cycle of the study population is a partial bivoltine (Kato, 2001). Adults of overwintering generation emerge in late April to early June; their offspring pupate and some individuals eclose in midsummer, but most of them diapause at the pupal stage and eclose the next spring. Low temperatures induce higher incidence of diapause under long-day conditions (Kato, 2000).

Aristolochia kaempferi, the host plant of *B. alcinous*, is commonly found on the forest floor and forest edge of coniferous plantations in the study area. Normally, this deciduous woody vine produces new leaves only from spring to early summer. Leaf density of this plant was variable, and individuals were sparsely distributed in the area with the highest deer density (Table 3.1).

3.2.2. Field experiments: the effects of deer on the leaf quality of host plant

Field experiments were conducted at Fudago, located in southern Boso peninsula, to examine the response of *A. kaempferi* to deer browsing. This experimental site was a plantation of Japanese cedar (*C. japonica*), and was located at the center of deer distribution in the Boso peninsula. The

estimated deer density at this site was 15 individuals / km^2 in 2007.

Experiment 1. Age-SPAD relationship

       The applicability of the Soil and Plant Analyzer Development (SPAD) value as an indicator of leaf age was tested to estimate leaf quality in the field. SPAD is an index of chlorophyll concentration and is known to be correlated with leaf age and age-related characteristics in various plant species (Hiyama et al., 2005; Marenco et al., 2009). SPAD values of *A. kaempferi* leaves with known age were measured with a chlorophyll meter (SPAD-502 Plus; Konica Minolta, Osaka, Japan), which allows rapid and nondestructive measurement, in an experimental cage (L × W × H = 7 × 2 × 3 m, covered with a 1-mm-mesh net) that excluded deer browsing. Experimental plants of *A. kaempferi* collected near the cage were planted in May 2009. I collected average-sized plants growing in coniferous plantations. Each plant was potted in forest soil and was watered by rainfall. Leaf age (in weeks) was identified by successive observations of individual shoots. The SPAD value of each leaf was measured once a week from April to May 2010 (the leaf flushing period of *A. kaempferi*) and then every 2 to 4 weeks until September 2010. The relationship between SPAD value and leaf age of 26 leaves of 11 potted plants was analyzed by a general additive model. All statistical analyses were performed with R 2.10.1 for Windows (R Development Core Team, 2009).

Experiment 2. Effects of deer exclusion on SPAD values and number of

leaves

Changes in SPAD values as well as number of leaves were measured inside and outside of the deer exclosures. Four pairs of exclosed and control plots were established in June 2010. The distances between pairs were > 3 m, and those between plots in a pair were < 1 m. The size of each plot was 0.6 × 0.45 m, which contained four potted *A. kaempferi*. In the experimental plots, pots were covered by a 5 × 5-cm-mesh wire net to prevent deer access. The SPAD values and the number of leaves per plant were measured in June 2010 and August 2011. The effects on SPAD values were analyzed by a randomized block ANOVA. Because the number of leaves showed a non-normal distribution, they were analyzed by generalized linear mixed models (GLMMs), which were analogous to a randomized block ANOVA. The response variable was the number of leaves, and the explanatory variables were treatment, block identity, and block × treatment interaction. Models were fitted with a log link function and a Poisson error structure. The effects of exclosures were tested by analysis of devience.

Experiment 3. Effects of simulated browsing on nutritional and physical quality of leaves

Because it was not possible to control the timing of natural browsing, qualitative changes of leaves after regrowth were measured by a simulated browsing experiment. Four pairs of treatment and control plots were located at distances of < 10 m between individual plots of the paired set and > 30 m between paired sets. Each 1 × 1 m plot contained naturally growing *A.*

kaempferi and was covered by a 10 × 10-cm-mesh net to prevent deer access. All leaves were clipped off the treatment plot plants in July 2007, and all leaves (5-14) were sampled from each plot 1 month later. Resprouting leaves were 2-3 weeks in age at sampling time. This clipping treatment was considered a similar level of disturbance that occurs from natural deer browsing. The C:N ratio and leaf toughness were measured to represent nutritional and physical quality, respectively. Leaf toughness was measured with a penetrometer (Sands and Brancatini, 1991). First, a leaf was sandwiched between two transparent plastic plates with a 3-mm-diameter hole and held horizontally. The tissue between major leaf veins was then pierced slowly by iron rod (2.6-mm-diameter) with a flat end. The maximum force used to pierce the leaf was measured with a digital force gauge (RX-5; Aikoh Engeneering, Osaka, Japan) attached to the iron rod. After measuring leaf toughness, leaves were oven dried at 60 °C for more than 48 h and then ground in a mortar. The C:N ratio of ground leaves was measured by a C:N analyzer (MT-700; Yanaco Analytical Instruments, Kyoto, Japan). The effects of simulated browsing on the C:N ratio and leaf toughness were analyzed by randomized block ANOVA.

3.2.3. Field surveys: regional variation in the frequency of young leaves and their utilization by the butterfly

Young leaf frequency of *A. kaempferi* and the oviposition pattern of *B. alcinous* were surveyed across the deer-density gradient. Six coniferous plantations, ranging from low to high in deer density, were selected as study

sites (Table 3.1). At each site, leaves of *A. kaempferi* found on the forest floor were chosen, SPAD values were measured, and the presence of *B. alcinous* eggs was recorded. Leaves were chosen from different shoots, and extremely small leaves (smaller than 4 cm^2) were excluded from the measurement. Leaves with eggs or the first instar larvae were regarded as oviposited leaves because first instar larvae of *B. alcinous* are not highly mobile. Surveys of leaves continued until 10 to 20 leaves having eggs or larvae were counted. The total numbers of leaves examined were from 117 to 401 at each site. Although the SPAD value is a continuous variable, a threshold value to distinguish between young and mature leaves was determined based on the response curve of SPAD against leaf age (see 3.3. Results). To account for possible arbitrariness, different threshold values (±5) were used, but the results did not change significantly (data not shown).

The effect of deer on the regional frequency of young leaves was estimated by a GLMM. The response variable was the age of leaves (young or mature), and the explanatory variables were the estimated deer density at each site as a fixed factor and site identity as a random factor. The effect of deer on utilization of young leaves by the butterfly was estimated by GLMM with the same explanatory variables. Whether the oviposited leaf was young or mature was treated as a response variable. Models were fitted with a logit ink function and a binomial error structure. The effects of deer were tested by analysis of deviance.

3.2.4. Laboratory experiment: the effects of leaf quality on the physiological

condition of butterfly

Study insect

　　Adult butterflies were collected at Fudago in 2006. Captive adult females were allowed to oviposit on *A. debilis* or *A. kaempferi* in transparent plastic cages. Larvae from three females were used for the experiment. Two of the adult females (A and B) were collected on 31 May, and another (C) was collected on 27 July.

Food plant

　　All larvae were fed *A. kaempferi* in this experiment. Almost all leaves were collected at Hachioji, Tokyo (35.63 °N, 139.24 °E; altitude 300-500 m; Honshu, Japan). Leaves were put into plastic bags immediately after collection and kept in a refrigerator until leaf toughness was measured. Although leaf toughness is a continuous variable, it was impossible to give leaves with exactly the constant toughness to each larva throughout the experimental period. Accordingly, I fed two levels of leaves with different toughness; leaves with a penetration force less than 1.5 N were categorized as soft, and those higher than 2.0 N were categorized as tough. C:N ratio differed significantly between tough and soft leaves ($F_{1,13}$ = 6.31, P = 0.03); the means (±SE) of tough and soft leaves were 18.70 ± 1.31 and 14.62 ± 0.88, respectively. C:N ratios of soft and tough leaves roughly corresponded to those of young leaves and mature intact leaves, respectively.

Rearing experiment: the effects of plant quality on B. alcinous

Larvae from each female were divided into two groups; one group was given soft leaves and the other was given tough leaves until pupation. All leaves given had been measured and categorized. The experimental period was 8 June – 31 August for larvae from female A, 8 June – 17 August from female B, and 5 August 4 November 2006 for larvae from female C. Larvae were reared in 900-ml transparent cups with moist paper under a constant temperature (20 °C) and photoperiod (16L:8D). Under these conditions, most of the individuals belonging to mountain populations (which use *A. kaempferi* as a host plant) enter diapause (Kato, 2000). The petiole of each leaf was wrapped with moist paper to avoid wilting. Leaves were exchanged for new ones before wilting or before being fully consumed. One to six larvae were reared in each cup depending on the larval instar, and all larvae were reared individually after the forth instar. The number of larvae reared in a cup did not differ between treatments. Larval duration, pupal weight, and pupal diapause were recorded for each larva. Leaf toughness may be deterrent, especially for younger larvae; thus, the number of larvae that died and survived until the third instar was recorded for each treatment. Larval duration was defined as the period from hatching to pupation. Pupal weight was measured with an electoric balance 1 or 2 days after pupation. At the same time, each pupa was identified as diapausing or non-diapausing by color (Kato, 2000); diapausing pupae are light brown, while non-diapausing pupae are bright yellow (Figure 3.2). When this discrimination was difficult, pupae that eclosed within 1 month under constant conditions (20 °C, 16L:8D) were regarded as non-diapausing.

The effects of leaf treatment on larval and pupal performance were analyzed with GLMM. Treatment was a fixed factor and female parent was a random factor. The response variables were larval duration, pupal weight, early larval survival rate, and the incidence of pupal diapause. The first two variables were assumed to have normal distributions with identity link functions. The latter two variables were assumed to have binomial distributions (dead or alive, diapausing or non-diapausing) with logit link functions. In the soft-leaf treatment, female C produced both diapausing and non-diapausing pupae, so the relationship between larval duration and the incidence of pupal diapause was analyzed by a logistic regression.

3.3. Results

3.3.1. Plant responses after browsing

The SPAD value of *A. kaempferi* increased with leaf age ($r^2 = 0.60$, $P < 0.001$; Figure 3.3). SPAD values showed a monotonic increase until about 7 weeks, and saturation occurred at SPAD values of approximately 35 to 45. Plants grown outside of the deer exclosures had fewer leaves and lower SPAD values than those inside in August (four replicated randomized complete block design; number of leaves: $\chi^2_1 = 7.79$, $P = 0.005$; SPAD: $F_{1,3} = 41.7$, $P = 0.008$; Figure 3.4). Number of leaves and SPAD values did not differ between inside and outside of the exclosures when the experiment started (number of leaves [mean ± 1SE]: inside [4.31 ± 0.373], outside [4.75 ± 0.393], $\chi^2_1 = 0.338$, $P = 0.560$; SPAD [mean ± 1SE]: inside [32.7 ± 0.689], outside [31.2 ± 1.49], $F_{1,3} = 0.443$, $P = 0.553$). Resprouting leaves after simulated

browsing had both lower C:N ratios and lower toughness compared to those in the unclipped control plots (C:N ratio: $F_{1,3} = 13.0$, $P = 0.04$; leaf toughness: $F_{1,3} = 26.1$, $P = 0.01$; Figure 3.5).

3.3.2. Regional variation in the frequency of young leaves and their utilization by butterfly

Because SPAD values of *A. kaempferi* showed saturation at about the age of 2 months with the level of about 35 (Figure 3.3), a SPAD threshold of 35 was set to distinguish between young and mature leaves. The frequency of young leaves varied regionally and increased with deer density ($\chi^2_1 = 6.12$, $P = 0.013$; Y = 1 / [1 + exp [2.3 -0.25 × deer density]]; Figure 3.11). Utilization of young leaves by *B. alcinous* was greater in high deer-density areas than in low-density areas ($\chi^2_1 = 13.63$, $P < 0.001$; Y = 1 / [1 + exp [2.1 -0.45 × deer density]]; Figure 3.11). Overall, the proportion of young leaves among all leaves on which *B. alcinous* oviposited was higher than the proportion of young leaves observed. In areas with high deer-densities, about 90 % of *B. alcinous* eggs were observed on young leaves.

3.3.3. Butterfly performance reared under different plant quality

Larval duration differed significantly between leaf toughness treatment ($F_{1,2} = 40.33$, $P = 0.02$; Figure 3.6), with larvae feeding on tough leaves having a longer larval period. Pupal weight did not differ between treatments ($F_{1,2} = 1.68$, $P = 0.32$; Figure 3.7). Survival rate until third instar was lower in larvae feeding on tough leaves than in larvae feeding on soft

leaves ($\chi^2_1 = 4.84$, $P = 0.03$; Figure 3.8). The incidence of pupal diapause differed significantly between the treatments ($\chi^2_1 = 7.91$, $P = 0.005$;.Figure 3.9). All but one pupa entered diapause in the tough-leaf treatment. In the soft-leaf treatment, larvae from female C had almost equal probabilities of becoming diapausing or non-diapausing pupae. I found a positive correlation between larval duration and the incidence of pupal diapause ($\chi^2_1 = 18.87$, $P < 0.001$; Figure 3.10), and diapause incidence drastically changed at a larval duration of around 50 days.

3.4. Discussion

3.4.1. The effect of deer on plant regrowth

Deer browsing stimulated the regrowth of *A. kaempferi*, which may compensate for aboveground biomass loss (Figure 3.4). Simulated browsing also induced compensatory growth of *A. kaempferi* and improved leaf nutritional and physical qualities for insects (Figure 3.5). Compensatory growth of host plant is one of the major mechanisms causing a positive indirect effect of large herbivores on insects (Haukioja et al., 1990). Sprouting ability of plants are known to evolve under environments subject to frequent disturbances (Bond & Midgley, 2003), and *A. kaempferi* also can grow in habitats where farmers frequently mow (Takagi & Miyashita, 2008). Plants with a high tolerance to disturbances are likely to transmit positive indirect effects via improved food quality for herbivores.

3.4.2. Geographic patterns of availability and utilization of young leaves

Young leaves were frequently observed in areas with high deer-densities (Figure 3.11). Although climate and/or productivity might have influenced young leaf frequency through phenological modification, correlation coefficients between deer density and latitude, altitude, canopy openness, and leaf density were all relatively low ($|\rho| < 0.35$) in the study areas. Therefore, regional variation observed in this study was most likely caused by deer herbivory. The proportion of young leaves on which butterflies oviposited was higher than that of young leaves to total leaves in each area (Figure 3.11). This suggests that adult female butterflies selectively oviposited on young leaves instead of mature ones. Previous studies have also shown a preference by phytophagous insects to compensatory shoots after mammalian browsing (Martinsen et al., 1998; Bailey & Whitham, 2006). Insects adapted to using young plant tissues may easily respond to occasional supplies of young leaves caused by browsing by large herbivores. Such insects are likely to be responsive to indirect effects mediated by plant quality.

In accordance with the preference by *B. alcinous* for young leaves, most eggs were observed on young leaves in high deer-density areas, while they were found on mature leaves in the lowest deer-density area (Figure 3.11). Young leaf recruitment after deer browsing significantly improved resource quality for *B. alcinous* at the regional level. Young leaf frequency among oviposited leaves showed a saturation pattern across a deer-density gradient. This means that even if current deer densities increase further in high deer-density areas, young leaf utilization by *B. alcinous* would not

change from the present level. Thus, rather than a positive influence resulting from improved leaf quality, heavy browsing pressure would result in resource depletion and cause a negative indirect effect on insect populations.

3.4.3. Indirect effects of deer browsing on butterfly

Soft leaves increased the survival rate of young larvae and shorten the larval stage (Figure 3.6, 3.8), suggesting positive effects of browsing-induced resprouts on the fitness components of *B. alcinous*. Lower survival rate in tough-leaf treatment was likely to be caused by the difficulty in chewing physically-defended leaves for small larvae, and longer larval duration may occur as the result of poor nutritional quality (low C:N ratio) of tough leaves. Tough leaves used in the rearing experiment showed similar levels of C:N ratio compared to unbrowsed leaves, whereas they were tougher than unbrowsed leaves observed in the field experiments. Therefore, positive effects of deer browsing on the survival of young larvae might be somewhat overestimated. Nevertheless, because a long larval duration may also reduce survivorship through the cumulative effects of predation risk (Haggstrom & Larsson, 1995) or parasitism (Benrey & Denno, 1997) under natural conditions, browsing-induced qualitative changes are likely to positively affect *B. alcinous* in larval stage. The pupal weight did not differ significantly between treatments (Figure 3.7). This suggests that there is no significant indirect effect on the reproductive output in adult stage, considering a general correlation between adult weight and its fecundity in

many insects (Honěk, 1993).

It is noteworthy that larvae from the same female increased their incidence of diapause in the tough-leaf treatment (Figure 3.9). In addition, larval duration and diapause induction showed a positive relationship (Figure 3.10). These suggested that leaf quality is certainly one of the diapause-inducing stimuli for *B. alcinous*. Although dietary quality is an important factor determining fitness in many phytophagous insects (Awmack & Leather, 2002), only a few studies have shown that diapause is induced by plant quality rather than by photoperiod or temperature (e.g., Tauber et al., 1986). The plastic diapause based on host quality observed in *B. alcinous* is probably due to the difficulty in predicting host plant quality using only photoperiod and temperature. As I reported above, *A. kaempferi* has an ability of regrowth after biotic or abiotic disturbance, which indicates that the phenology of host plant of *B. alcinous* is flexible and changes in response to occasional disturbance. Therefore, the ability of using host plant quality as a diapause-inducing stimulus may be adaptive for *B. alcinous* using *A. kaempferi* subject to disturbance. The conditional diapause response to plant quality may also have an ecological significance in the context of plant-mediated indirect interactions. Diapause affects fitness of a focal individual as well as the annual population growth rate. When pupae do not enter diapause in summer, population has twofold reproductive opportunities per year in comparison with a diapausing population. This means non-diapausing populations grow rapidly for increasing populations, while declines considerably for decreasing populations. The improvement of

leaf quality by deer browsing is expected to lead to a higher population growth and lower diapause induction of *B.alcinous*. If *B. alcinous* achieves a positive population growth, the plastic diapause enhances positive indirect effects mediated by plant quality.

I focused on positive trait-mediated effect of deer on insects, but negative effects may also prevail under high deer-density conditions. The experiment showed a decrease in the number of leaves under high browsing pressure (Figure 3.4a). Although regional leaf densities of *A. kaempferi* did not show conspicuous relationship to the current deer densities, leaf density was lowest in the area with the highest deer density (Table 3.1; Fudago). Because deer is likely to reduce plant biomass cumulatively over the years, long-term research is needed to clarify the relative importance of quality- and biomass-mediated indirect effects on insects in the future. Another possible process causing negative effect is incidental omnivory on insects by large herbivores (Gómez & González-Megías, 2007b). However, as most of the deer browsing on *A. kaempferi* occurred in April to May when *B. alcinous* is pupae or adults (unpublished data), incidental omnivory is unlikely to be important.

3.4.4. Concluding remarks

In this chapter, three possible processes of indirect effects of deer browsing on *B. alcinous* were demonstrated: (1) Compensatory regrowth of host plants may improve larval survival rate; (2) Compensatory regrowth of host plants may result in low incidence of pupal diapause, which provides an

opportunity to yield additional generation in summer; (3) Biomass reduction

in host plant may cause food depletion. The experimental approach is

effective in clarifying such processes of indirect effects (Gómez &

González-Megías, 2007b). However, as many of the earlier experiments were

conducted in a single area with a given herbivore density, predicting "dose

response" of insects to deer density is impossible. Our field survey across a

deer-density gradient suggested that positive effects saturate in high

deer-density areas. To predict the response of insects to future changes in

densities or future range expansions in large herbivores, it appears

promising to conduct surveys across a density gradient of large herbivores as

well as experimental approach.
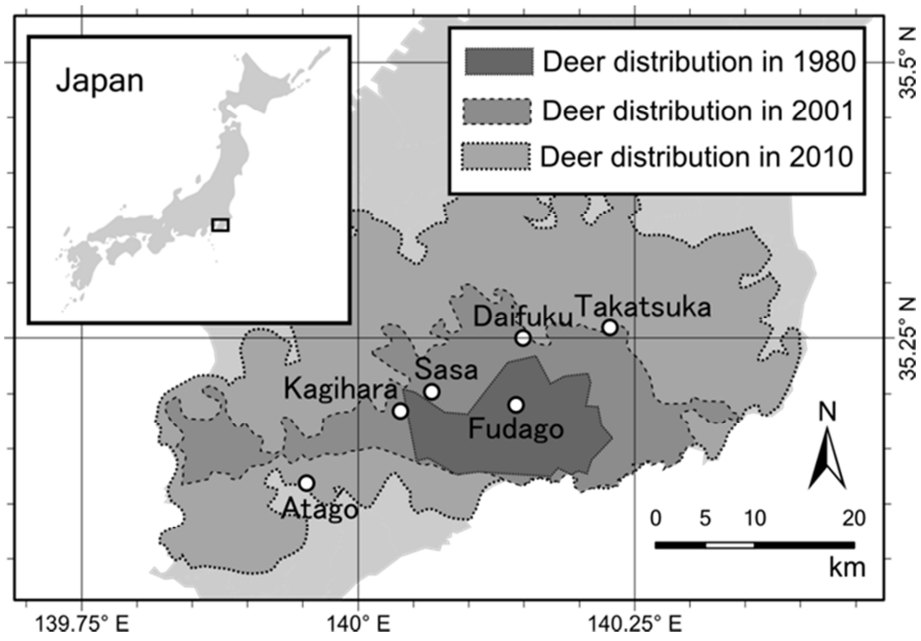
Figure 3.1.

Study sites for field surveys and the deer distribution area in 1980, 2001, and 2010
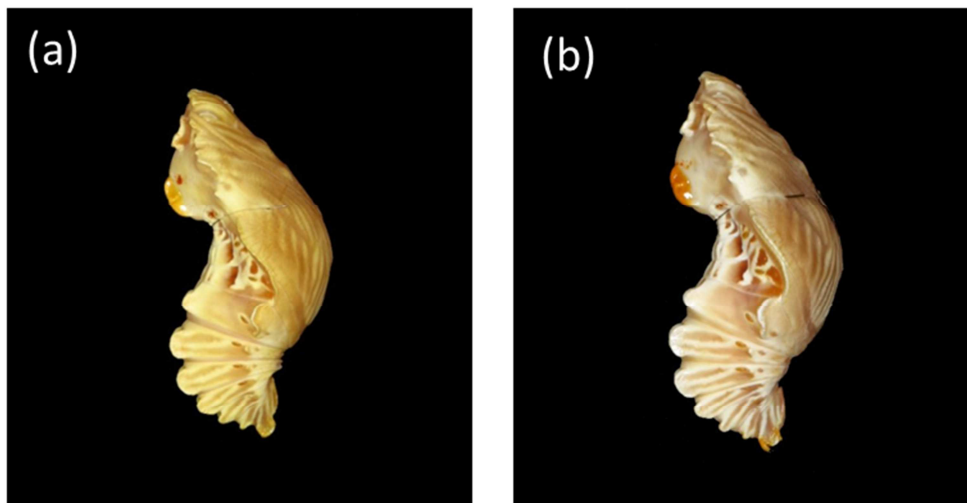
Figure 3.2.

The pupal color dimorphism of *B. alcinous*. (a) Non-diapausing. (b) Diapausing
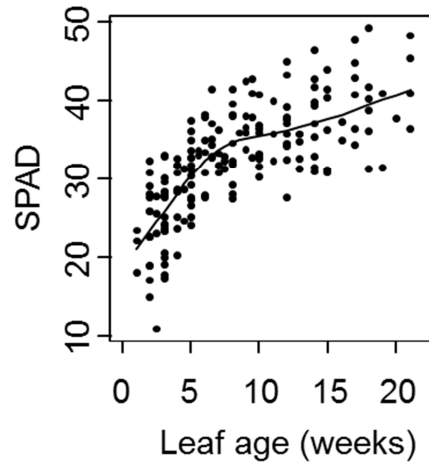
Figure 3.3.

Relationship between SPAD value and age in weeks of *A. kaempferi* leaves in

a deer-exclosed cage. The solid curve was estimated by a general additive

model.
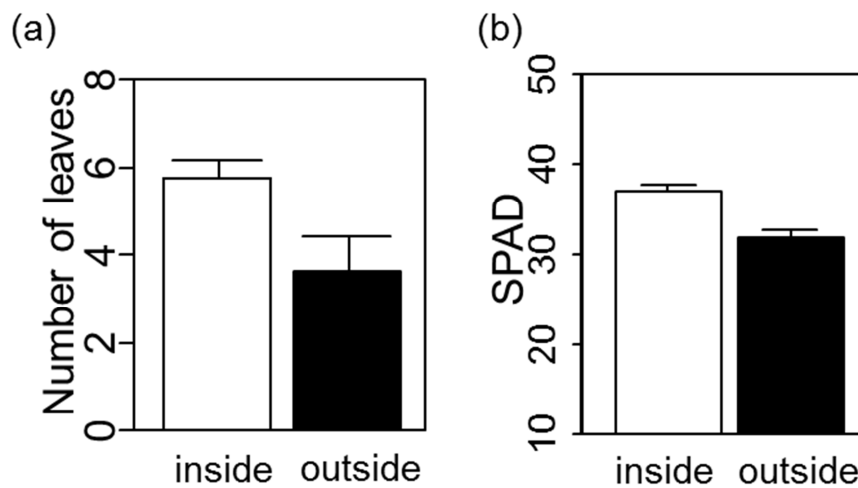
Figure 3.4.

Number of leaves per year (a) and SPAD values (b) of *A. kaempferi* inside

and outside of four deer exclosures (means + 1SE are shown) in August
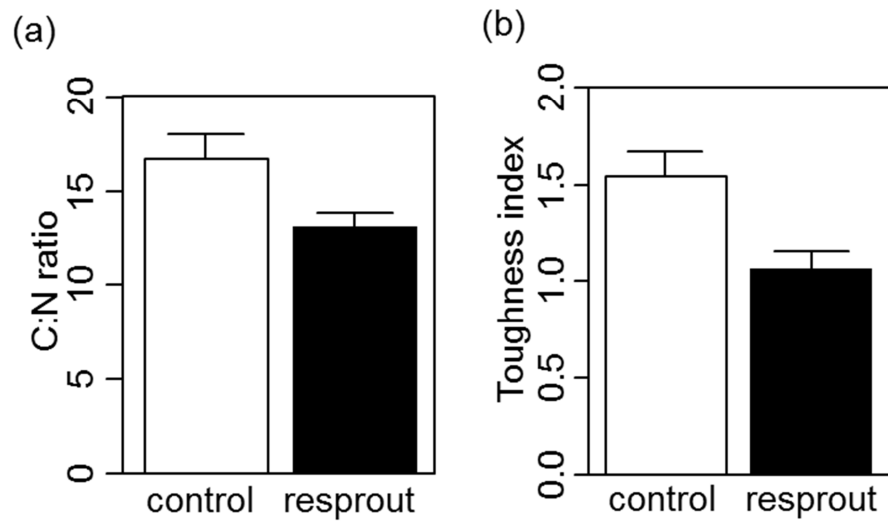
Figure 3.5.

C:N ratio (a) and toughness (b) of *A. kaempferi* leaves under four pairs of control and simulated browsing (resprout) treatments (means + 1SE are shown).
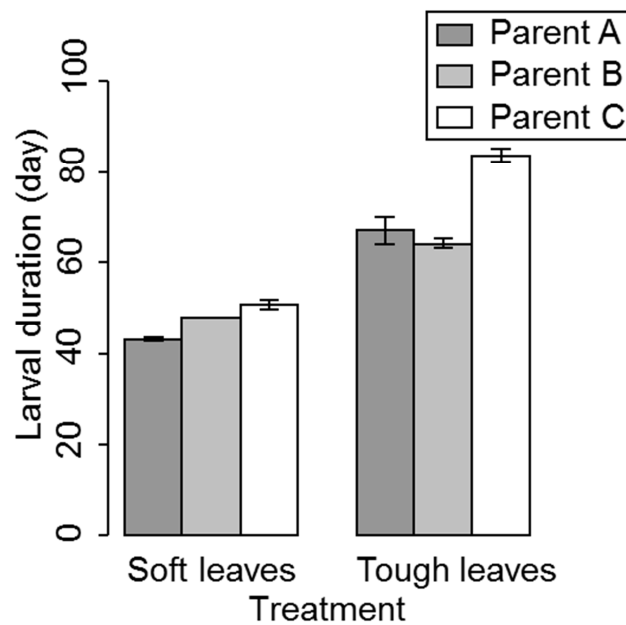
Figure 3.6.

The effect of leaf toughness on larval duration. Data represent mean ±1SE.
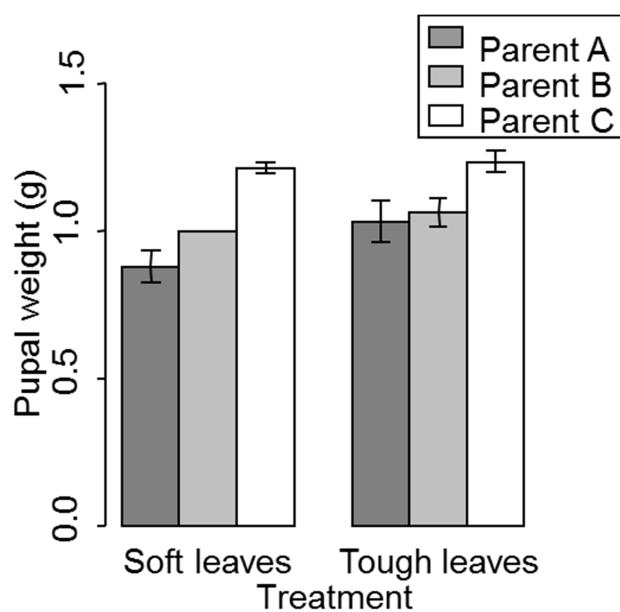
Figure 3.7.

The effect of leaf toughness on pupal weight. Data represent mean ±1SE.
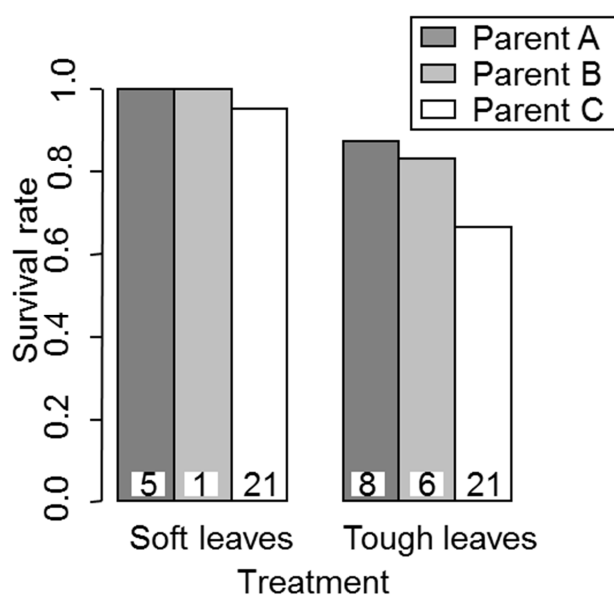
Figure 3.8.

The effect of leaf toughness on survival rate of young larvae. Sample sizes

are given in each bar.
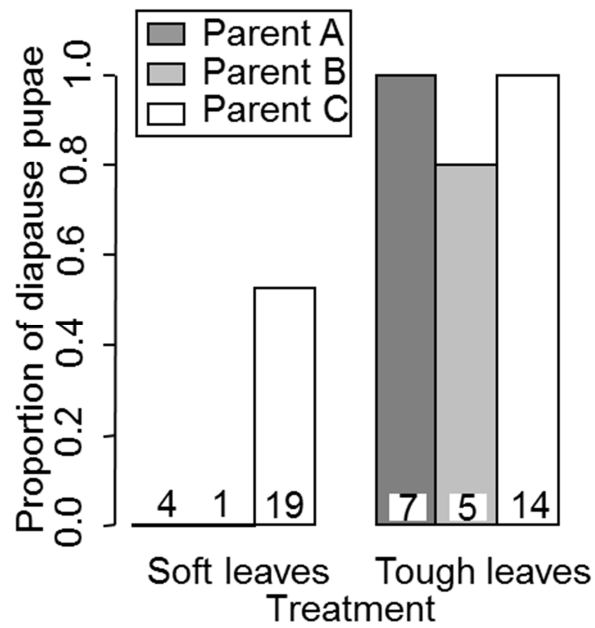
Figure 3.9.

The effect of leaf toughness on incidence of pupal diapause. Sample sizes are gives in each bar.
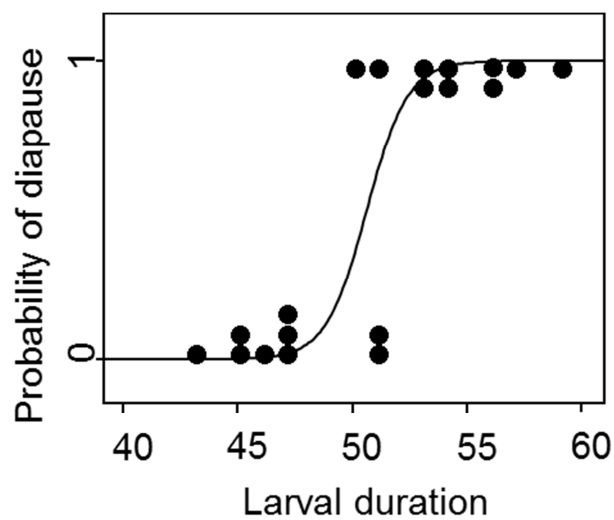
Figure 3.10.

The relationship between larval duration and incidence of pupal diapause, larvae from parent C in soft-leaf treatment. Each dot represents individials.
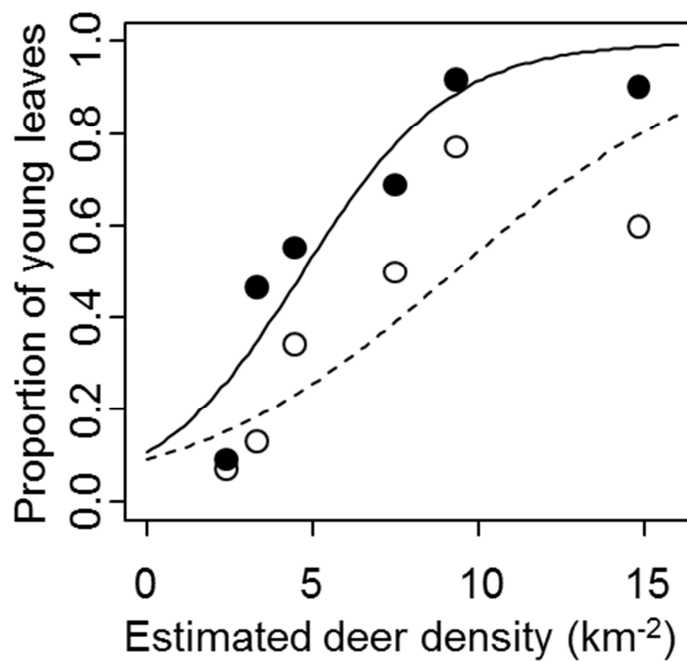
Figure 3.11.

Geographic variation in the proportion of young leaves to total number of leaves observed (white circle, dashed curve) and those oviposited on (black circle, solid curve) by adult butterflies B. *alcinous*. Numbers of leaves observed and oviposited are 378, 117, 401, 235, 305, 117, and 10, 12, 16, 20, 15, 11, respectively (descending order of deer density).

Table 3.1.

Characteristics of the study sites for the field surveys. Deer densities were estimated by pellet counts in 2007. Leaf densities were measured by 3 replicated $1 \times 20m2$ transect around each study site. Because leaf densities varied locally, minimum to maximum densities were shown.

Site name	Latitude (°N)	Longitude (°E)	Altitude (m)	Deer density (km^{-2})	Canopy openness (%)	Leaf density ($10m^{-2}$)	Site area (ha)
Fudago	35.20	140.14	151	14.8	12.8	0-5.5	1.5
Kagihara	35.18	140.04	187	9.4	18.1	7-55.5	0.25
Sasa	35.20	140.07	167	7.5	12.0	0-32	1.5
Takatsuka	35.26	140.23	123	4.5	13.7	4.5-42.5	0.75
Daifuku	35.25	140.15	164	3.4	14.9	4.5-101.5	0.75
Atago	35.12	139.95	204	2.4	11.2	1.5-33.5	0.75

Chapter 4: Time scale dependency of the indirect effect of deer on *B. alcinous*

4.1. Introduction

Deer browsing induced regrowth of *A. kaempferi*, which may leads to a positive indirect effect on *B. alcinous* (Chapter 3). As well as the positive effect, the negative indirect effect mediated by resource reduction was also suggested. Both positive indirect effects mediated by plant quality and negative indirect effects mediated by plant quantity are widely known in "large herbivore-plant- phytophagous insect" systems (Gómez & González-Megías, 2007a). Although these two processes should act simultaneously in nature, their relative strength were rarely estimated. Estimating indirect effects without clarifying background processes may lead to erroneous interpretations or predictions.

When plants are browsed by large herbivores, there should be a large difference in time-scale between biomass reduction and plastic trait changes. As described in chapter 2, quantity mediated indirect effects in plants cannot be detected in short term experiments. In contrast, compensatory regrowth after browsing can be detected in short-term exclosure experiments or simulated browsing (Chapter 3). When large herbivores expand their geographic distribution range, the important process of indirect interactions may differ between the central areas of the distribution and peripheral areas. To understand the indirect effects of the changes in herbivore density, we need to identify the temporal scale to which the density or traits of

transmitter plants respond to herbivory.

There are mainly two approaches to consider multiple time-scales in previous studies. One is an experiment-based approach, which is made by observations of insect densities at treatments with different large herbivore densities (e.g., Gómez & González-Megías, 2007b). Experiments can detect causal relationship and give detail information on changes in transmitter plants over time. The other is a pattern-based approach, which compares insect densities in areas with different histories of grazing pressure by large herbivores (e.g., Martin et al., 2009). Pattern studies have an advantage in estimating the effects operating in larger spatio-temporal scales. For highly mobile species (such as large Lepidoptera) or slowly responding transmitter plants, it is difficult to estimate indirect effects by experimental approach while pattern studies could estimate indirect effects. It should be in mind that the estimations from observations often depend on the past history of focal populations of large herbivores. If there are large regional variations in the dynamics of large herbivores and have large variations in histories among regions, pattern-based approach could effectively estimate time-scale dependency of indirect effects of large herbivores.

The density of sika deer population in the Boso Peninsula was estimated for 15 years at each 1-km^2-grid (Figure 4.1). As this population has been expanding its distribution range, areas with different browsing histories are available. In addition, several areas show decreasing tendency in deer densities due to intensive population control. Thus, plants or insects are likely to have experienced different browsing history both in short-term

and long-term in different areas, which makes it possible to estimate quantity- and quality-mediated effects.

This chapter examines time-scale dependency of the indirect effects of deer on the swallowtail butterfly mediated by quantity and quality of host plants. The following hypotheses were addressed. (1) Aboveground biomass of *A. kaempferi* decreases in areas suffering deer browsing pressure for a long period, and the percentage of young leaves increases with current deer-densities. (2) The density of *B. alcinous* increases with increasing amount and quality of leaves of *A. kaempferi*. (3) indirect effect of deer on *B. alcinous* is positive with respect to quality mediated effect by plants in short-term, whereas negative with respect to quantity mediated effects in long-term.

4.2. Materials and Methods

4.2.1. Study system

Field surveys were conducted on the Boso Peninsula, central Japan (Figure 4.2). Details were described in Chapter 3.

4.2.2. Host plant

The quantity and quality of *A. kaempferi* were measured at the 30 study sites in 9 regions where *Criptomeria japonica* or *Chamaechyparis obtuse* dominated (Figure 4.2). Regions were defined based on the geographical proximity to butterfly census routes (describe in 4.2.3). Quantities of leaves were measured in mid or late June, 2010. All leaves

within transect (20×1 m^2) were counted. Each transect was located at the

forest interior more than 10-m apart from forest edge. Qualities of leaves

were measured as SPAD value, which was correlated with leaf age (Chapter

3). SPAD values were measured by a chlorophyll meter (SPAD-502 Plus;

Konica Minolta, Osaka, Japan). Fifteen leaves were measured in each study

site in early July 2010. Extremely small leaves (smaller than 4 cm^2) were

excluded from the measurement. Canopy openness was measured by the

photos taken by the fish-eye converter (UWC-1628; FIT Corporation, Nagano,

Japan), and openness were estimated by CanopOn2

(http://takenaka-akio.cool.ne.jp/etc/canopon2/). Topographic Wetness Index

(TWI; Sørensen et al., 2006) was calculated from the 15-m grid digital

elevation model by the following formula,

$$TWI = ln\ (A\ /\ tan\ B\ )$$

Where A is the flow accumulation area and B is the slope of each grid. TWI

was calculated by ArcGIS 10.0 (ESRI, 2010). Leaf density, young leaf

frequency, and environmental conditions of each study sites are listed in

Table 4.1.

4.2.3. Butterfly density

Densities of *B. alcinou*s were estimated by route census. Occurrence

of adult butterflies showed a bimodal annual pattern with peaks in April to

June and July to August. As adults observed in July to August are

non-diapausing individuals, and represent a portion of the total population,

adult countings were conducted in May when all individuals in a population

eclosed. Censuses were conducted at 4 routes in 2008, 8 routes in 2009 and 2010 (Figure 4.2). The length of each route was 6.5 km, and 1.5 hour (10:30-12:00 or 14:00-15:30) was spent for each census by walk on sunny or cloudy days. Adult butterflies within < 10 m from the observer were counted. Most of the butterflies observed were wandering. Although some individuals were visiting flowers, those individuals were not concentrated in a particular route. Therefore, observation count reflected the relative densities of *B. alcinous* at each site. Three to four censuses per season were conducted at each route, and the maximum count was used as an indicator of density at the site of the year. In June 2010, leaf density and percentage of young leaves of *A. kaempferi* growing on the forest floor and number of large individual of *A. kaempferi* growing around the census route were measured as environmental variables. Quantity and quality of leaves found on the forest floor were measured at every 500m of each route (a total of 13 points per route). Measurements were performed by the method as described in 4.2.2. Mean values of quantity and quality of leaves at 13 points at each route were used as indicators of plant quantity and quality. *Aristolochia kaempferi* grows also on the forest edge, which are sparsely distributed at the side of a road. As plants growing on the forest edge have tall vegetation height, these plants are unlikely to suffer from deer browsing. *Byasa alcinous* uses both plants growing on the forest floor and the forest edge. Large individuals of *A. kaempferi* (> 1.5m in height) growing on the forest edge were counted within < 10m from each census route. Because both *A. kaempferi* and *B.alcinous* mainly inhabit forested areas, census routes with a high forest cover were

expected to have more butterflies. To adjust for this bias, forest coverage of each route was calculated from vegetation map (J-IBIS; Ministry of the Environment, Japan) by generating a 1500m-radius buffer from a focal census route. Temperature at each census time was obtained from AMeDAS (Automated Meteorological Data) at Kamogawa (35.1 °N, 140.1 °E). Butterfly counts and environmental variables are listed in Table 4.2.

4.2.4. Deer density

Deer density was estimated by the pellet count data obtained previously from 1997 to 2011 (Chiba Prefecture, 2008; Asada, 2009; 2011). The density of each 1-km^2 grid was estimated for each year, following the method by Suzuki et al., (2008). Estimated density at each study site is described in Table 4.3.

4.2.5. Statistical analyses

Indirect effects of deer on the butterfly mediated by plant quantitative and qualitative change were analyzed by the following two steps. First, generalized linear mixed models (GLMMs) were used to estimate the direct effects of deer on plants, and that of plants on the butterfly. To reduce the number of explanatory variables model selection was performed based on Akaike Information Criterion (AIC). Next, indirect effects of deer on the butterfly were analyzed by a path analysis including variales selected from GLMM, and parameters were estimated by a hierarchical Bayesian model. The time-scale of the effect of deer on plant

quantity or quality was estimated by the degree of carry-over effects. A high rate of carry-over means longer scale responses; effects on plant cumulatively increase with successive deer browsing history.

Estimation of direct effects by GLMMs

Response variables and explanatory variables are listed in Table 4.4. First, the effects on quantity or quality of *A. kaempferi* were analyzed. Plant quantity (leaf density) was assumed to have negative binomial distribution, and binomial data of plant quality (young or mature leaves) was assumed to have binomial distribution. Link functions were 'log' and 'logit', respectively. The threshold value for classifying young and mature leaves was set at SPAD = 35 as in chapter 3. Two types of deer-density indices were used; short-term deer density (the average of 2010-2011) and long-term deer density (the average of 1997-2011). Region ID was treated as a random factor, and deer density, openness, and TWI were treated as fixed factors for GLMMs. Models were constructed with all combinations of fixed factors (8 models for short and long term effects). Models with the lowest AIC were compared among time-scales. Second, the effect on the density of *B. alcinous* was analyzed. Number of individual counted was assumed to have Poisson distribution, with log link function. Because each census route included two or three year observations, all models included "year" as a fixed factor and route ID as a random factor. The effect on the butterfly density was analyzed by GLMM which includes 7 explanatory variables i.e., year, route ID, quantity and quality of *A. kaempferi*, number of large plants, elevation, and

temperature (total of 32 models). Logarithm of forest cover (%) was also included in the model as an offset value. Because how the butterfly densities response to resource quantity and quality was unknown, two different measures were used for representing the effects of quantity and quality of *A. kaempferi*; (1) log (leaf density) and logit (% young leaves), which assume that plant quantity and quality affect additively; (2) log (young leaf density) and log (mature leaf density), which assume that the availability of high and low quality leaves affect additively. All GLMMs were performed by glmmADMB library in R 2.12.1 (R developing core team, 2010).

Estimation of indirect effects by Bayesian model

A hierarchical Bayesian model was constructed for estimating the cumulative effects of deer on plant quantity and quality, the relative strength of quantity- and quality-mediated effects, and time-scale dependency of the total indirect effect. Model structure is shown graphically in Figure 4.4 and parameters are listed in Table 4.5. The model consists of (1) the description of cumulative effects of deer on plant quantity and quality and (2) the description of quantity- and quality-mediated effects of plant on the butterfly densities.

The cumulative effects of deer on plants were described as

$$\beta_{deer} \times \frac{\sum(\alpha^t \cdot deer_t)}{\sum \alpha^t}$$

β_{deer}: per capita effect of unit deer density (1 individual / km^2)

α: carry-over effect to next year [0-1]

t: year (0, 1, 2, …, t_{max})

deer$_t$: deer density t years ago

When deer colonizes into novel habitats, the cumulative effect increases with years after colonization with a saturation pattern (Figure 4.3). Higher α values indicate slow saturation patterns and lower α values indicate fast saturation patterns. Saturation level of effects at year t_{max} is β_{deer}. Variables selected from GLMM analyses were included in the Bayesian model.

The effect of deer on leaf density of *A. kaempferi* at region i site j $[L_{ij}]$ was modeled as the following.

$$L_{ij} \sim Negbin\ (p_{ij}, r);\ \epsilon_{1i} \sim Normal\ (0, \theta_1)$$

$$p_{ij} = r/(l_{ij} + r)$$

$$\log(l_{ij}) = \beta_{01} + \beta_{op1} \times OPEN_{ij} + \beta_{twi1} \times TWI_{ij} + \beta_{deer1} \times \frac{\sum(\alpha_1{}^t \cdot deer_{ijt})}{\sum \alpha_1{}^t} + \epsilon_{1i}$$

Leaf density $[L_{ij}]$ is assumed to be affected by canopy openness $[OPEN_{ij}]$, TWI $[TWI_{ij}]$, and cumulative effect of deer. Leaves are assumed to be counted as a negative Binomial process. Random process error for leaf density at region i was ϵ_{1i}.

The effect of deer on young leaf frequency of *A. kaempferi* $[Y_{ij}]$ was modeled as the following,

$$Y_{ij} \sim Bin\ (y_{ij}, 15);\ \epsilon_{2i} \sim Normal\ (0, \theta_2)$$

$$\text{logit}\ (y_{ij}) = \beta_{02} + \beta_{op2} \times OPEN_{ij} + \beta_{twi2} \times TWI_{ij} + \beta_{deer2} \times \frac{\sum(\alpha_2{}^t \cdot deer_{ijt})}{\sum \alpha_2{}^t} + \epsilon_{2i}$$

Young leaves out of 15 leaves $[Y_{ij}]$ were assumed to be observed by binomial process with probability y_{ij}. Explanatory variables were the same as in the model of leaf density, but the carry-over rate $[\alpha_2]$ was assumed to be different.

Observations of *B. alcinous* in route i year k [N_{ik}] were modeled as following,

$$N_{ik} \sim Poisson\ (\mu_{ik})$$

$$\log(\mu_{ik}) = \gamma_{ik} + \beta_{temp} \times TEMP_{ik} + \log(FOREST_i)$$

$$\gamma_{ik} = \beta_k + \beta_l \times \log(l_{ij}) + \beta_y \times \text{logit}\ (y_{ij}) + \beta_{elev} \times ELEV_i$$

Butterfly abundance was assumed to be dependent on leaf density [l_{ij}], % young leaf [y_{ij}] and elevation [$ELEV_i$]. Number of observations is assumed to increase with forest cover at each site [$FOREST_i$].

Estimation of posterior distributions of parameters was conducted by WinBUGS1.4. Prior distribution of α was assigned as Uniform distribution (min=0, max=1), that of β was uninformative Normal distribution (mean=0, variance=10^5), and that of θ was inverse of Gamma distribution (shape=0.001, scale=0.001). Three MCMC (Markov Chain Monte Carlo) chains were run with 200000 iteration (100000 burn in), and 1000 posterior probabilities were sampled per chain. Convergence of parameters was judged from R-hat value (Brooks and Gelman, 1998).

The strength of each indirect effect was expressed as,

$$\text{quantity-mediated indirect effect} = \beta_l \times \beta_{deer1} \times \frac{\sum(\alpha_1{}^t \cdot deer_t)}{\sum \alpha_1{}^t}$$

$$\text{quality-mediated indirect effect} = \beta_y \times \beta_{deer2} \times \frac{\sum(\alpha_2{}^t \cdot deer_t)}{\sum \alpha_2{}^t}$$

The current year effect of x individuals of deer was described as, ($deer_0 = x, deer_1 = deer_2 = \cdots = deer_{t_{max}} = 0$)

$$\text{quantity-mediated indirect effect}_{current} = \beta_l \times \beta_{deer1} \times \frac{(1 - \alpha_1) \times x}{1 - \alpha_1{}^{(t_{max}+1)}}$$

$$\text{quality-mediated indirect effect}_{current} = \beta_y \times \beta_{deer2} \times \frac{(1 - \alpha_2) \times x}{1 - \alpha_2^{(t_{max}+1)}}$$

Cumulative effect of x individuals of deer was ($deer_0 = deer_1 = \cdots = deer_{t_{max}} = x$)

$$\text{quantity-mediated indirect effect}_{cumulative} = \beta_l \times \beta_{deer1} \times x$$

$$\text{quality-mediated indirect effect}_{cumulative} = \beta_y \times \beta_{deer2} \times x$$

The total strength of indirect effect was calculated by sum of these effects. t_{max} of these analyses was set at 13 (data from 1997 to 2010/2011). The current and 13-year cumulative quantity-mediated, quality-mediated, and the total indirect effects were compared.

4.3. Results

Direct effects of deer density on A. kaempferi

The leaf density of *A. kaempferi* was not affected by short-term deer density, but negatively affected by long-term deer density (Table 4.6). Canopy openness and TWI affected positively on leaf density. All models with AIC values lower than that of the null model included long-term deer density in their explanatory variables. The proportion of young leaves increased with deer-density (Table 4.7). Models including deer density had much lower AIC than the null model. Current-deer density model showed the lowest AIC. Canopy openness and TWI also affected positively. All of the good models (ΔAIC<2) included deer density as an explanatory variable.

Direct effects of host plant on B. alcinous

The density of *B. alcinous* was associated with quantity and quality

of *A. kaempferi* (Table 4.8); positive effects were observed in areas with high leaf density and high percentage of young leaves. Whichever types of quantity and quality indicators used had little effects on AIC values, the availability of young and mature leaves affected butterfly densities positively and negatively, respectively. The explanatory variable of the best model included plant quantity and quality as well as elevation and temperature, with the latter two having positive effects on the butterfly density. Number of large plant individuals appeared unimportant.

Carry-over effects of deer on leaf density and young leaf frequency

Estimated posterior distribution of each variable was listed in Table 4.9. Similar to the results of GLMM, deer density negatively affected leaf density and positively affected percentage of young leaves (β_{deer1}: -0.234 ± 0.087; β_{deer2}: 0.129 ± 0.038; mean $\pm$ SD). Carry-over rates showed different posterior distributions between the effects on plant quantity and quality (Figure 4.5). Carry-over rate for the effect on leaf density [α_1] had a high posterior probability at high values of α (median: 0.93), with 90.4% of the posterior distribution within a range of [0.7-1.0]. In contrast, carry-over rate for the effect on the young leaf percentage [α_2] did not show a clear peak in the posterior distribution (median: 0.621). The posterior distributions of saturation patterns in the cumulative effects of deer browsing are shown in Figure 4.6. The indirect effect mediated by plant quantity had a high carry-over rate, showing relatively slow saturation pattern.

Time-scale dependence of quantity- and quality-mediated indirect effects

Quality-mediated process affected positively, and quantity-mediated process affected negatively on butterfly density (Figure 4.7). For the current year effect, positive quality-mediated effect was stronger than quantity-mediated effects, and thus the total effect showed a positive trend (Median: 0.0199; 95%CI: [-0.0076, 0.0773]). The cumulative effect was stronger than the current year effect in both processes, and quantity-mediated negative effect cumulatively increased. The median strength of quantity- and quality-mediated effects increased cumulatively 8.57-fold and 2.62-fold compared to the current year effect. The total effect did not show a clear pattern (Median: 0.00559; 95%CI: [-0.0670, 0.0905]) because negative and positive processes had almost similar strengths and balanced each other.

4.4. Discussion

Deer exhibited a negative effect on leaf density and a positive effect on the proportion of young leaves of *A. kaempferi,* which led to negative and positive indirect effects on *B. alcinous*. Plant quantity and quality showed different time-scale responses.

Leaf density of *A. kaempferi* was not correlated with short-term deer density but negatively correlated with long-term deer density (Table 4.6). As a result, plant quantity was affected cumulatively by historical deer browsing. About 90% of the posterior distribution of carry-over rate for the effect on leaf density [α_1] was > 0.7. This means that plants may recover less

than 30 % of biomass which had lost by the last year browsing. The ability of

A. kaempferi to recover its biomass loss may be dependent on habitat

conditions, and plants growing on forest floor under closed canopy may need

long time for compensating biomass loss after herbivory due to low

productivity.

The proportion of young leaves increased with current deer density,

which is consistent with the findings in chapter 3 that shot-term simulated

browsing increased in young leaf frequency. The carry-over rate for the effect

on the percentage of young leaves [α_2] showed a lower value than that for the

effect on leaf density [α_1]. However, [α_2] exhibited a wide credible interval of

the posterior distribution (95%CI: [0.032, 0.943]). If young leaf frequency

responds to only current year browsing, $\alpha_2 = 0$ is expected. There were

mainly two reasons why such a wide posterior distribution was estimated.

First, limited information on deer density resulted in a low precision of the

estimation. There were high temporal auto-correlations between $deer_t$ and

$deer_{t+1}$. Highly auto-correlated data cannot estimate carry-over effects with

a high precision. Furthermore, the data includes many missing values

because the survey on deer pellet density at each site were conducted every 2

years (half of the entire deer distribution range was surveyed in a year; Table

4.3). The effect of missing values may not be so problematic, because

long-term effects were estimated by the period of 1997-2011. In contrast, as

information in recent years is important for estimating the short-term effect,

the existence of missing values may be more serious. Second, past browsing

history may affect current browsing pressure. Browsing pressure on a

specific plant may depend not only on current herbivore density but also on relative abundance of the plant. *Aristolochia* plants contain Aristolochic acids which deter vertebrate herbivory (Nishida & Fukami, 1989) and may have relatively low palatability to deer. Browsing pressure is expected to increase in several years after deer colonization when other palatable plants decreased. In such a case, a relatively high value of α_2 will be estimated due to indirect effect of the past browsing pressure.

Because plant quantity and quality showed different time-scale responses, strength of per capita effect of deer on *B. alcinous* varied with time-scale. The total indirect effect did not show specific trends at the long time-scale because positive and negative effects canceled each other. In contrast, the current-year effect had a positive influence on butterfly density because quality-mediated positive effect prevailed over quantity-mediated negative effect. These patterns are consistent with general trends found in chapter 2, i.e., short-term studies tended to show positive effects. Taking into account the range expansion of deer distribution in the Boso Peninsula, indirect effect of deer on *B. alcinous* is likely to have positive influences only at the recently colonized area, and do not appear to have much effect under equilibrium conditions. Because *A. kaempferi* occasionally grows taller than deer accessible height, reductions in plant density on the forest floor may not lead to sever resource depletion for *B. alcinous*. Plants growing on the forest floor may be important for non-diapausing individuals because leaves of large plants toughen in summer. As reported in chapter 3, young leaf availability was restricted in low deer-density areas in summer. Young leaf

supplementation by deer browsing may be important for survival of offsprings of non-diapausing butterflies.

In this chapter, I assumed that a certain proportion of a given effect is carried to the next year, and coefficient of carry-over rate could be regarded as an indicator of time-scale dependency. Here, I propose an advantage of quantitative estimation of time-scale dependency, which is based on the assumption of specific processes. Process-oriented model is easy to be combined with the data from experimental approach. In the model used in this chapter, time-scale dependency was determined by carry-over rate which is a measurable parameter by exclosure experiments. Estimation of carry-over rate by pattern approach and experimental approach will complement each other. As discussed above, estimation of carry-over rate for short-term response by field patterns tends to have a low precision, whereas short-term manipulative experiments have a high precision of estimation of carry-over rate. Combining estimated parameters from experiments with models derived from large-scale patterns makes it possible to predict large-scale ecological responses that are difficult to predict by only small-scale experiments. Although I estimated parameters only from pattern data, experimentally estimated parameters can be used as prior probabilities of parameters, which could lead to a high precision of the estimation. There also remained some problems with this approach. The effect of large herbivores on plant sometimes shows non-linear responses. For example, the rate of change in plant biomass differ between responses against herbivore introduction and exclusion (Oloffson, 2006). Because deer population in the

Boso Peninsula is still expanding, the analyses presented here may have detected processes occurring in the increasing phase of deer. When the population density decreases by human control, models sincorporating plant recovery process should be constructed. In such a case, combining the pattern approach and the experimental approach that can detect the response of plants to decreasing deer will be promising.
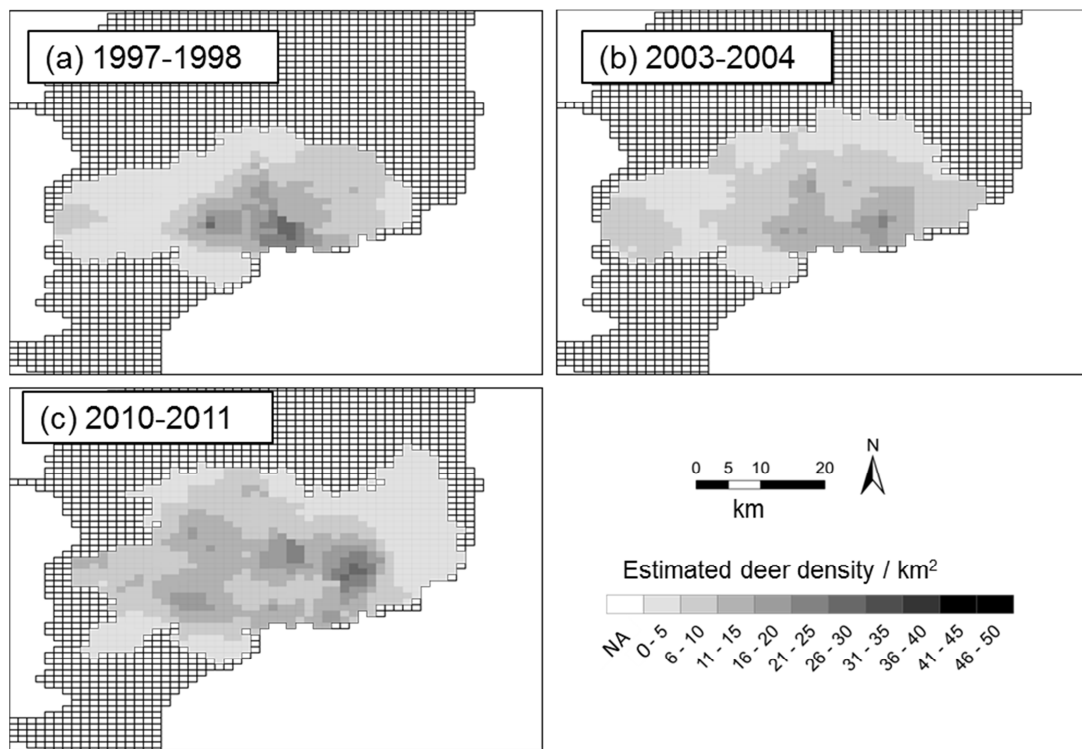
Figure 4.1.

Geographic distributions of deer density in the Boso Peninsula in 1997-1998
(a), 2003-2004(b), and 2010-2011(c).
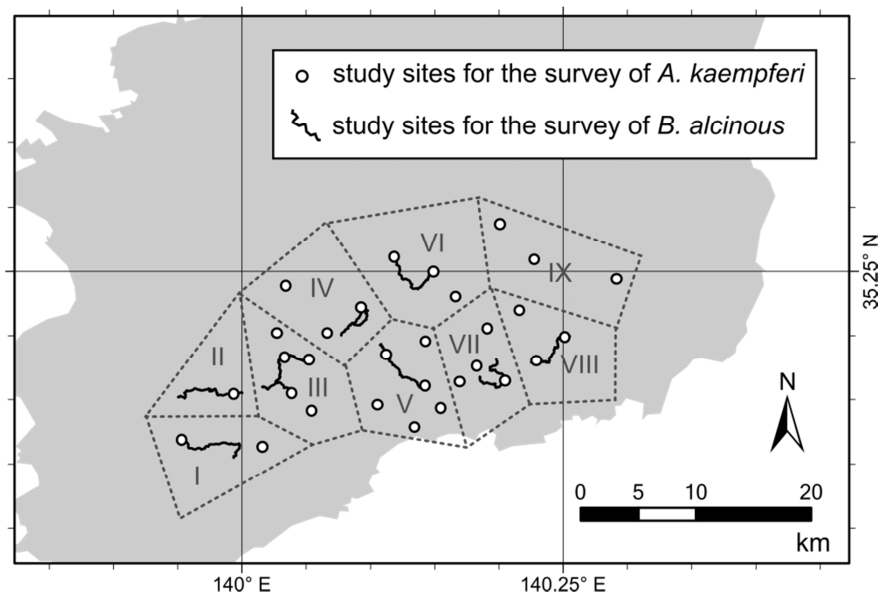
Figure 4.2.

Study sites for the field surveys.
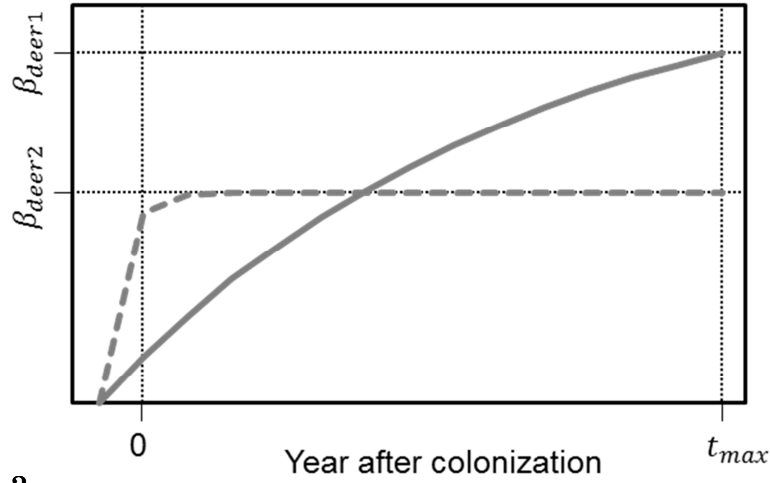
Figure 4.3.

Example of saturation patterns of the effects of deer on plant. $\alpha_1 = 0.9$ (solid

line), $\alpha_2 = 0.1$ (dashed line)
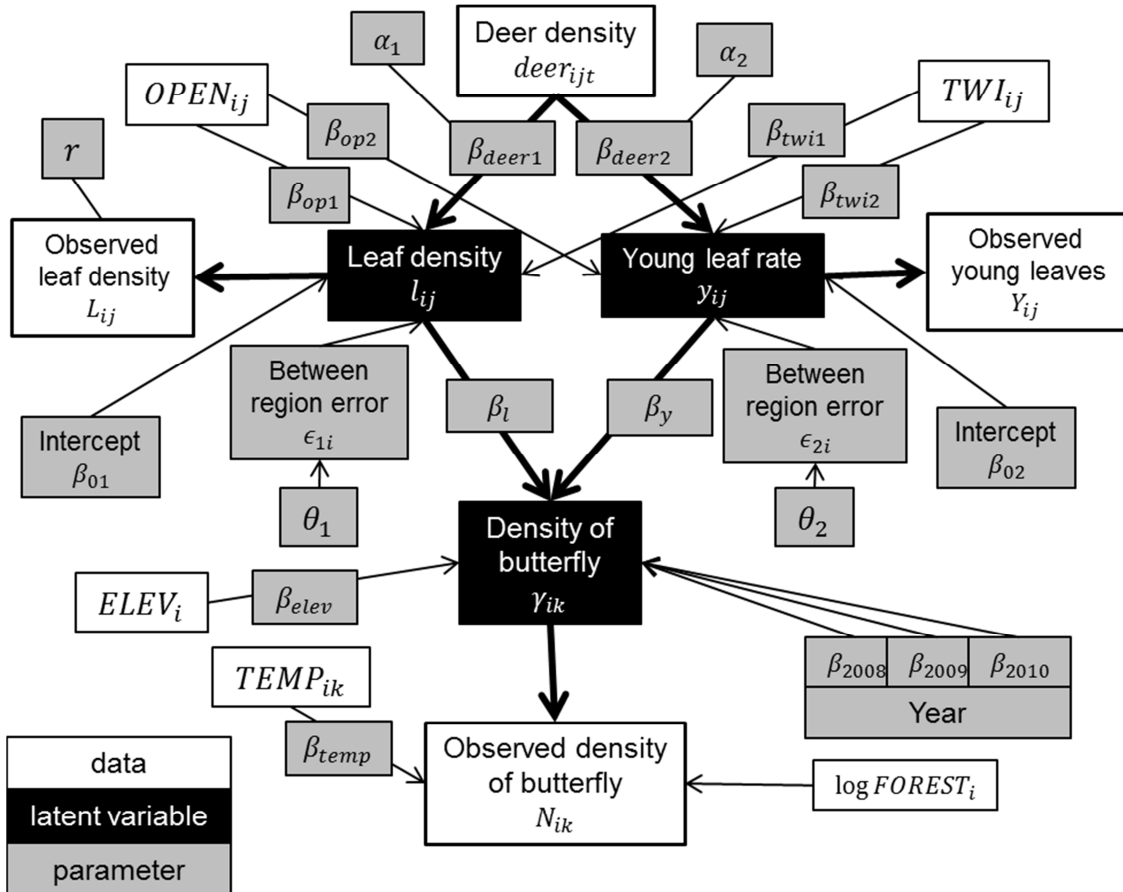
Figure 4.4.

Graphical view of the model structure.
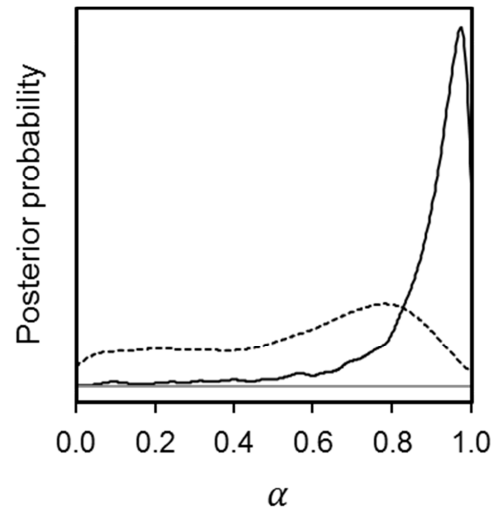
Figure 4.5.

Estimated posterior distributions of α_1(solid) and α_2 (dash) .
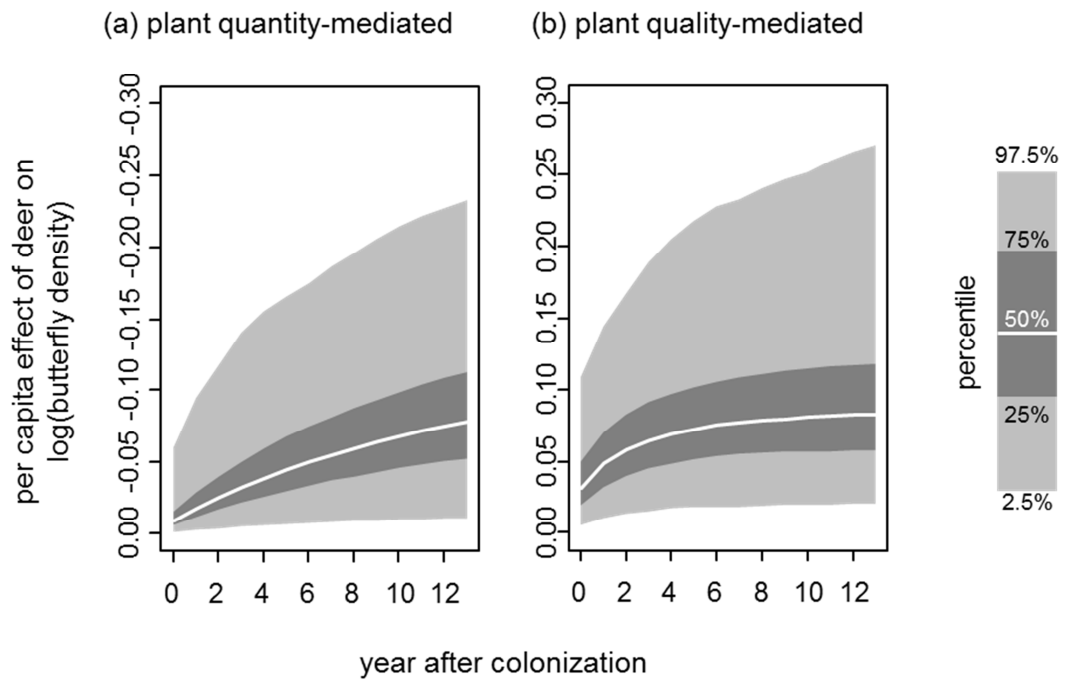
Figure 4.6.

Cumulative effects of quantity-mediated effect (1), quality-mediated effect

(2) of deer on *B. alcinous*. Posterior probabilities are shown.
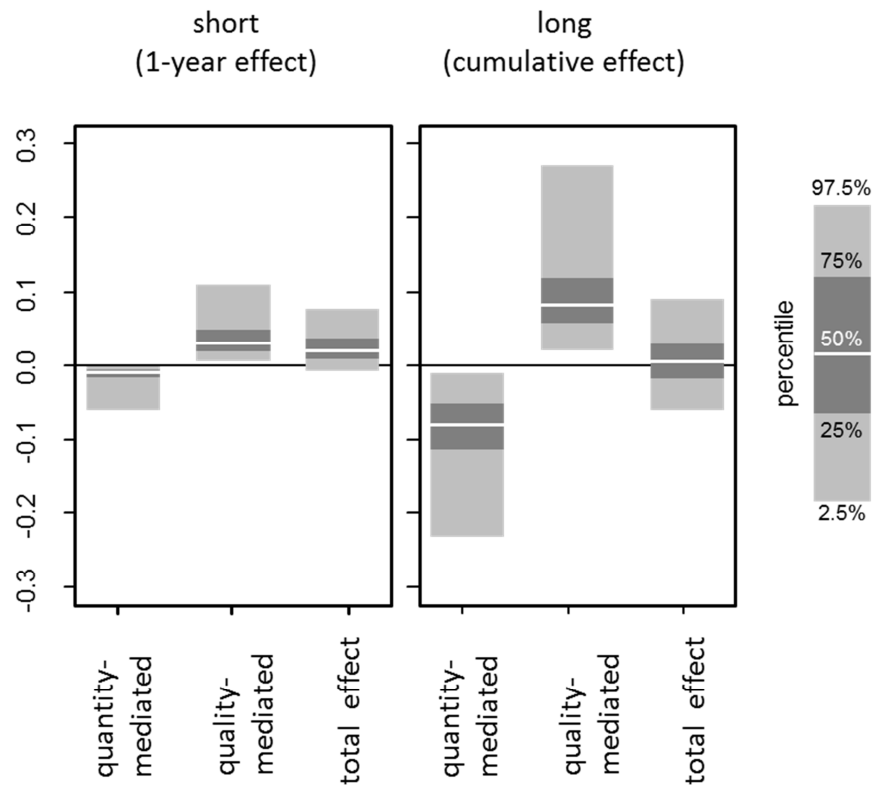
Figure 4.7.

Current (a) and cumulative (b) indirect effects of deer on *B. alcinous* density.

Posterior probabilities of quantity- and quality-mediated effects and total

effect are shown.

Table 4.1.

Environmental conditions of study sites

Sitename	Region	Number of leaves (20m^{-2})	Young leaves (/15 leaves)	Canopy openness (%)	TWI
Amatsu	VIII	0	2	4.43	5.83
Arakine	IX	9	5	3.60	7.18
Atago	I	3	0	11.37	7.78
Bodai	III	11	9	9.70	6.62
Daihuku1	III	203	9	15.05	8.20
Daufuku2	III	67	1	6.27	6.71
Dentyusan	VI	14	5	6.43	7.11
Fudago	III	5	6	13.77	9.01
Fukurokura	III	11	10	12.55	9.84
Futama	III	3	8	10.07	7.76
Godai	III	0	5	21.62	6.74
Kagihara	VIII	17	11	6.60	6.78
Kaisho	VIII	10	12	12.03	8.38
Kinone	IV	13	6	8.18	6.14
Kimitsukameyama	V	53	13	8.50	6.86
Kiyosumi	III	11	4	9.02	6.77
Mineoka	I	9	6	7.02	7.20
Mituishi	V	0	6	8.70	7.40
Nonozuka1	VI	0	12	7.65	6.70
Nonozuka2	VI	8	12	5.87	7.62
Odagaku	VIII	34	11	15.40	8.43
Osaka	V	76	5	10.55	8.09
Otadai	III	9	11	9.83	6.78
Seiwa1	VIII	14	8	11.67	9.75
Seiwa2	VIII	3	10	9.29	7.02
Tabina	VIII	64	11	8.90	7.48
Takatuka	IX	63	5	10.37	6.93
Tukiide	IX	85	3	7.15	8.09
Utiura	VIII	0	8	6.65	8.22
Yoro	VIII	27	8	7.75	6.63

Table 4.2.

Environmental conditions of census routes

Route	Number of butterflies			Temperature			Mean number of leaves $(20m^{-2})$	Mean number of young leaves $(20m^{-2})$	Number of large plant $(6.5km^{-1})$	Elevation (m)	Forest cover (%)
	2008	2009	2010	2008	2009	2010					
I	-	8	12	-	23.8	24.0	18.5	7.3	24	292.7	62.7
II	-	12	7	-	21.3	23.7	6.5	3.2	16	244.8	77.5
III	29	17	11	24.5	15.5	23.5	9.2	8.2	22	234.2	89.7
IV	-	7	6	-	21.6	25.0	6.8	4.5	8	188.5	78.7
V	8	16	8	22.8	21.6	23.1	3.3	1.7	9	281.8	94.3
VI	11	22	2	18.6	18.1	19.3	29.4	12.5	6	205.0	85.8
VII	-	11	4	-	21.2	15.2	10.3	4.6	30	148.9	83.8
VIII	11	10	3	19.6	18.8	17.1	9.9	4.8	11	168.2	78.6

Table 4.3.

Deer density dynamics in each study site

Sitename	Region	year														
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Amatsu	VIII	24.0	14.0	16.0	10.6	15.7	19.2	13.8	7.3	13.8	15.7	12.0	7.3	12.6	7.9	9.8
Arakine	IX	NA	NA	NA	NA	NA	NA	NA	3.9	12.4	5.6	4.4	4.3	NA	4.0	NA
Atago	I	4.3	NA	4.1	NA	3.9	NA	4.2	NA	4.5	NA	5.0	NA	5.5	NA	5.0
Bodai	III	17.6	NA	15.7	NA	11.3	NA	13.3	NA	8.9	NA	12.1	NA	10.7	NA	8.5
Daihuku1	III	NA	4.5	NA	4.4	NA	13.9	NA	5.2	5.4	5.4	6.8	5.8	NA	8.1	NA
Daufuku2	III	5.1	3.9	6.4	4.1	5.5	8.6	7.7	4.6	5.2	4.5	6.5	5.8	7.7	7.6	9.0
Dentyusan	VI	NA	7.9	NA	9.7	NA	11.2	NA	7.4	13.2	17.4	15.5	13.7	NA	15.8	NA
Fudago	III	14.2	11.0	13.3	10.1	13.3	20.4	12.9	8.2	11.8	13.1	13.1	10.7	35.5	11.3	18.7
Fukurokura	III	22.2	NA	12.8	NA	11.4	NA	12.2	NA	12.2	NA	15.5	NA	11.8	NA	15.9
Futama	III	27.6	NA	16.4	NA	15.4	NA	13.2	NA	15.3	NA	13.2	NA	11.4	NA	10.3
Godai	III	16.0	NA	12.0	NA	9.5	NA	13.6	NA	9.7	NA	13.7	NA	15.5	NA	12.3
Kagihara	VIII	12.1	NA	8.3	NA	9.2	NA	7.7	NA	8.3	NA	12.5	NA	14.0	NA	12.0
Kaisho	VIII	22.5	11.8	16.9	10.4	18.3	18.1	14.9	8.5	13.5	16.5	11.6	10.4	21.8	10.8	11.5
Kinone	IV	7.0	NA	4.5	NA	7.9	NA	6.8	NA	9.4	NA	8.9	NA	14.8	NA	17.5
Kimitsukameyama	V	6.8	NA	5.1	NA	8.5	NA	7.3	NA	6.8	NA	8.7	NA	14.1	NA	13.6
Kiyosumi	III	22.8	12.2	13.0	9.2	13.9	18.2	12.2	7.3	11.9	15.1	10.7	7.9	10.8	7.2	10.4
Mineoka	I	3.9	NA	3.9	NA	3.9	NA	4.3	NA	3.9	NA	3.9	NA	10.0	NA	6.2
Mituishi	V	10.8	NA	8.3	NA	8.2	NA	10.2	NA	7.8	NA	9.8	NA	14.1	NA	11.8
Nonozuka1	VI	NA	9.5	NA	11.1	NA	13.1	NA	13.5	15.2	21.4	21.6	18.3	NA	25.2	NA
Nonozuka2	VI	NA	6.8	NA	8.5	NA	10.9	NA	10.2	13.6	19.1	23.3	21.5	NA	23.7	NA
Odagaku	VIII	17.0	NA	12.4	NA	9.4	NA	11.3	NA	11.3	NA	15.4	NA	18.8	NA	13.8
Osaka	V	3.9	NA	4.1	NA	6.4	NA	5.5	NA	6.0	NA	6.7	NA	13.2	NA	15.8
Otadai	III	NA	7.1	NA	7.5	NA	16.1	NA	7.6	10.5	9.1	10.6	8.7	NA	11.1	NA
Seiwa1	VIII	9.7	NA	5.4	NA	8.7	NA	8.7	NA	8.0	NA	11.0	NA	14.4	NA	14.0
Seiwa2	VIII	23.6	NA	12.1	NA	8.8	NA	8.3	NA	9.2	NA	16.2	NA	13.9	NA	13.2
Tabina	VIII	5.1	NA	4.2	NA	7.1	NA	8.0	NA	5.8	NA	8.2	NA	12.3	NA	13.1
Takatuka	IX	NA	NA	NA	NA	NA	NA	NA	4.1	5.4	6.6	6.6	5.4	NA	5.7	NA
Tukiide	IX	NA	NA	NA	NA	NA	NA	NA	4.1	5.1	4.4	4.9	4.6	NA	4.3	NA
Utiura	VIII	25.8	10.4	18.9	11.1	16.1	18.8	17.2	14.9	17.7	18.5	18.1	18.6	18.1	18.4	12.0
Yoro	VIII	NA	8.8	NA	10.2	NA	17.1	NA	8.9	12.9	15.2	13.4	12.7	NA	13.8	NA

Table 4.4.

Variables used in GLMMs

#LEAVES	Number of leaves
%YOUNG	Proportion of young leaves
SDEER	Short-term average deer density (2010-2011)
LDEER	Longt-term average deer density (1997-2011)
OPEN	Canopy openness
TWI	Topographic Wetness Index
N	Number of butterflies
log#LEAVES	log (number of leaves)
logit%YOUNG	logit (proportion of young leaves)
log#YOUNG	log (number of young leaves)
log#MATURE	log (number of mature leaves)
LARGE	Number of large plant
ELEV	Elevation
TEMP	Temperature at survey time
YEAR	Observation year

Table 4.5.

Parameters and data used in a Bayesian model.

Parameters

β_{01}	Intercept for the leaf density
β_{02}	Intercept for the proportion of young leaves
β_{op1}	The effect of canopy openness on leaf density
β_{op2}	The effect of canopy openness on proportion of young leaves
β_{twi1}	The effect of TWI on leaf density
β_{twi2}	The effect of TWI on proportion of young leaves
β_{deer1}	Per capita effect of deer density on leaf density
β_{deer2}	Per capita effect of deer density on proportion of young leaves
β_{2008}	Intercept for the butterfly density in 2008
β_{2009}	Intercept for the butterfly density in 2009
β_{2010}	Intercept for the butterfly density in 2010
β_l	The effect of log(leaf density) on butterfly density
β_y	The effect of logit(proportion of young leaf) on butterfly density
β_{elev}	The effect of elevation on butterfly density
β_{temp}	The effect of temperature on butterfly density
α_1	Carry over rate of the effect of deer on leaf density
α_2	Carry over rate of the effect of deer on proportion of young leaves
r	Size parameter for negative binomial distribution of leaf density
ϵ_{1i}	Random process error for leaf density at region i
ϵ_{2i}	Random process error for proportion of young leaves at region i
θ_1	Hyperparameter for variation of ϵ_{1i}
θ_2	Hyperparameter for variation of ϵ_{2i}

Latent variables

l_{ij}	Leaf density at region i site j
y_{ij}	Young leaf rate at region i site j
γ_{ik}	Buterfly density at region i in year k

Data

L_{ij}	Observed leaf density at region i site j
Y_{ij}	Observed young leaf frequency at region i site j
N_{ik}	Buttterfly counts at region i in year k
$OPEN_{ij}$	Canopy openness at region i site j
TWI_{ij}	Topographic Wetness Index at region i site j
$deer_{ijt}$	Estimated deer density at region i site j, t years ago
$TEMP_{ik}$	Temperature at the observation time for region i year k
$ELEV_i$	Mean elevation of region i
$FOREST_i$	Forest cover around the census route at region i

Table 4.6.

Factors affecting the leaf density of *A. kaempferi*. Estimated value and standard error of each coefficient are shown.

Selected models (ΔAIC < 2) and null model are listed.

(1) short time-scale

	formula	ΔAIC	Intercept Estimate	SE	SDEER Estimate	SE	OPEN Estimate	SE	TWI Estimate	SE
1	#LEAVES ~ 1	254.982	3.1373	0.35357	NA	NA	NA	NA	NA	NA

(2) long time-scale

	formula	AIC	Intercept Estimate	SE	LDEER Estimate	SE	OPEN Estimate	SE	TWI Estimate	SE
1	#LEAVES ~ LDEER + OPEN	243.492	3.704	0.745	-0.015	0.003	0.107	0.065	NA	NA
2	#LEAVES ~ LDEER + TWI	243.976	2.140	1.781	-0.016	0.003	NA	NA	0.357	0.238
3	#LEAVES ~ LDEER	244.256	4.747	0.465	-0.015	0.003	NA	NA	NA	NA
4	#LEAVES ~ LDEER + OPEN + TWI	245.178	2.760	1.853	-0.015	0.003	0.076	0.085	0.170	0.306
	#LEAVES ~ 1	254.982	3.137	0.354	NA	NA	NA	NA	NA	NA

Table 4.7.

Factors affecting the young leaf frequency of *A. kaempferi*. Estimated value and standard error of each coefficient are shown. Selected models (ΔAIC < 2) and null model are listed.

(1) short time-scale			Intercept		SDEER		OPEN		TWI	
	formula	AIC	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
1	%YOUNG ~ SDEER	168.82	-0.979	0.245	0.006	0.001	NA	NA	NA	NA
2	%YOUNG ~ SDEER + TWI	170.17	-1.599	0.803	0.006	0.001	NA	NA	0.089	0.109
3	%YOUNG ~ SDEER + OPEN	170.22	-1.178	0.352	0.006	0.001	0.023	0.029	NA	NA
4	%YOUNG ~ SDEER + OPEN + TWI	171.88	-1.594	0.800	0.006	0.001	0.017	0.031	0.067	0.116
	%YOUNG ~ 1	184.81	-0.116	0.205	NA	NA	NA	NA	NA	NA
(2) long time-scale			Intercept		LDEER		OPEN		TWI	
	formula	AIC	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
1	%YOUNG ~ LDEER	171.35	-1.086	0.315	0.009	0.002	NA	NA	NA	NA
2	%YOUNG ~ LDEER + TWI	172.51	-1.773	0.815	0.008	0.002	NA	NA	0.098	0.107
3	%YOUNG ~ LDEER + OPEN	172.80	-1.286	0.417	0.008	0.002	0.023	0.031	NA	NA
4	%YOUNG ~ LDEER + OPEN + TWI	174.31	-1.776	0.816	0.008	0.002	0.015	0.033	0.080	0.115
	%YOUNG ~ 1	184.81	-0.116	0.205	NA	NA	NA	NA	NA	NA

Table 4.8.

Factors affecting the density of *B. alcinous*. Estimated value and standard error of each coefficient are shown.

Selected models (ΔAIC < 2) and null model are listed.

(1) total leaf density and % young leaves

formulas	AIC	Intercept Estimate	Intercept SE	log(#LEAVES) Estimate	log(#LEAVES) SE	logit(%YOUNG) Estimate	logit(%YOUNG) SE	LARGE Estimate	LARGE SE	ELEV Estimate	ELEV SE	TEMP Estimate	TEMP SE
1 N ~ log#L + logit%Y + ELEV + TEMP + YEAR	120.21	0.377	0.759	0.304	0.114	0.243	0.073	NA	NA	0.003	0.002	0.046	0.029
2 N ~ log#L + logit%Y + TEMP + YEAR	120.48	0.670	0.748	0.277	0.116	0.243	0.074	NA	NA	NA	NA	0.063	0.027
3 N ~ log#L + logit%Y + ELEV + YEAR	120.73	1.213	0.546	0.264	0.110	0.236	0.071	NA	NA	0.004	0.002	NA	NA
4 N ~ log#L + logit%Y + LARGE + ELEV + TEMP + YEAR	122.05	0.422	0.758	0.294	0.116	0.227	0.081	0.004	0.010	0.003	0.002	0.043	0.030
5 N ~ log#L + logit%Y + LARGE + ELEV + YEAR	122.12	1.175	0.538	0.253	0.112	0.208	0.079	0.008	0.010	0.004	0.002	NA	NA
Nj ~ 1 + YEAR + (1 | site)	126.97	2.770	0.163	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

(2) young and mature leaf density

formulas	AIC	Intercept Estimate	Intercept SE	log(#YOUNG) Estimate	log(#YOUNG) SE	log(#MATURE) Estimate	log(#MATURE) SE	LARGE Estimate	LARGE SE	ELEV Estimate	ELEV SE	TEMP Estimate	TEMP SE
1 N ~ log#Y + log#M + ELEV + TEMP + YEAR	120.52	0.568	0.723	0.466	0.124	-0.149	0.073	NA	NA	0.003	0.002	0.045	0.029
2 N ~ log#Y + log#M + ELEV + YEAR	120.92	1.362	0.508	0.432	0.121	-0.155	0.072	NA	NA	0.004	0.002	NA	NA
3 N ~ log#Y + log#M + TEMP + YEAR	121.02	0.887	0.706	0.438	0.126	-0.160	0.075	NA	NA	NA	NA	0.062	0.027
4 N ~ log#Y + log#M + LARGE + ELEV + YEAR	122.19	1.306	0.501	0.394	0.128	-0.127	0.079	0.009	0.010	0.004	0.002	NA	NA
5 N ~ log#Y + log#M + LARGE + ELEV + TEMP + YEAR	122.26	0.609	0.716	0.439	0.134	-0.133	0.079	0.005	0.010	0.003	0.002	0.041	0.030
N ~ 1 + YEAR	126.97	2.770	0.163	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 4.9.

Posterior distributions of parameters estimated by Bayesian model.

Parameter	mean	sd	quantile					Rhat
			2.50%	25%	50%	75%	97.50%	
β_{01}	5.62	0.905	3.865	5.03	5.614	6.193	7.485	1.001
β_{02}	-1.563	0.44	-2.526	-1.833	-1.536	-1.261	-0.774	1.001
β_{op1}	0.191	0.364	-0.538	-0.046	0.184	0.435	0.9	1.003
β_{op2}	0.035	0.12	-0.2	-0.048	0.034	0.116	0.271	1.001
β_{twi1}	0.229	0.296	-0.312	0.03	0.211	0.411	0.836	1.006
β_{twi2}	0.049	0.102	-0.153	-0.02	0.05	0.12	0.245	1.001
β_{deer1}	-0.235	0.085	-0.401	-0.289	-0.238	-0.18	-0.064	1.001
β_{deer2}	0.127	0.037	0.063	0.102	0.124	0.149	0.211	1.001
β_{2008}	1.354	1.412	-1.695	1.032	1.618	2.009	2.523	1.007
β_{2009}	1.482	1.395	-1.535	1.198	1.743	2.124	2.651	1.008
β_{2010}	0.712	1.408	-2.285	0.395	0.984	1.351	1.913	1.008
β_l	0.427	0.435	0.067	0.225	0.342	0.525	1.37	1.006
β_y	0.791	0.513	0.196	0.484	0.683	0.954	2.128	1.009
β_{elev}	0.227	0.18	-0.088	0.109	0.217	0.332	0.618	1.001
β_{temp}	0.151	0.1	-0.041	0.084	0.15	0.217	0.348	1.001
α_1	0.878	0.153	0.376	0.851	0.929	0.97	0.998	1.001
α_2	0.559	0.271	0.034	0.337	0.616	0.79	0.946	1.001
r	0.709	0.226	0.356	0.549	0.677	0.836	1.237	1.003
θ_1	0.089	0.224	0.001	0.005	0.02	0.076	0.611	1.002
θ_2	0.139	0.171	0.004	0.042	0.086	0.179	0.578	1.001

Chapter 5: General discussion

In this study, I examined time-scale dependency of density- and trait-mediated indirect effects in the "large herbivore-plant-phytophagous insect" system. In chapter 2, I reviewed studies on indirect effects in large herbivore-plant-phytophagous insect system, and found the directions of indirect effects to be dependent on the transmitter plant types and time-scale of studies. In general, negative effects prevailed, but studies on indirect effects mediated by tree species conducted in short time-scales had a tendency to show positive influences. In such situations, plant quality-mediated positive effects were fairly strong because plant quantity-mediated effects occurred infrequently in trees and difficult to be detected in short-time scales. In chapter 3, I estimated the process of trait-mediated indirect effect of deer on *B. alcinous* mediated by *A. kaemoferi*. Deer browsing induced regrowth of *A. kaempfri*, and improved the quality of leaves. Young leaf availability increased with deer density, and young leaf utilization by butterflies also increased. *Byasa alcinous* larvae fed by good quality leaves increased their survival rate, growth rate, and decreased the incidence of pupal diapause. Consequently, the combination between plastic response of *A. kaempferi* to deer browsing and plastic response of *B. alcinous* to food quality may lead to positive trait-mediated effects. However, the positive effect may saturate and the negative effect may become prevail under high deer-density conditions. In chapter 4, I estimated the time-scale dependency of deer browsing to *B. alcinous* mediated by quantity and quality

of *A. kaempferi*. Leaf density of *A. kaempferi* cumulatively decreased in response to successive deer herbivory, while percentage of young leaves increased with current deer density. Plant quantity-mediated effects had a negative influence and quality-mediated process had a positive influence. Total indirect effects showed a positive trend in short-term due to relatively strong trait-mediated effect, but they showed no specific trends in long-term because trait- and density- mediated effects canceled each other.

According to these results, I obtained the following two important findings: (1) indirect effects of large herbivores on insects mediated by host plants exhibit a time-scale dependency; (2) this context dependent pattern arises from the combination of cumulative effect of density-mediated process and rapid effect of trait-mediated process. There have been no empirical evidence supporting the time-scale dependency of the relative importance of density- and trait-mediated effects. One of the major problems of trait-mediated indirect effect in earlier studies is a scarcity of long-term studies (Werner & Peacor, 2003). Time-scale dependency of indirect effects presented here implies the necessity of long-term studies. It is noteworthy that this pattern is not consistent with general patterns reported in trophic cascade systems. Meta analyses for trophic cascade system showed no significant relationship between the strength of indirect effects and time-scale of studies (Menge, 1997; Schmitz et al., 2000; Shurin et al., 2002; Bell et al., 2003). Why does this inconsistency arise? Time-scale dependency is caused by the mismatch between response times of rapid trait-change and cumulative density-change. If the cumulative effects of density-change had

been as rapid as the trait-change, the relative strength of density- and trait-mediated effects would not have depended on the duration of experiments. In trophic cascade studies, transmitter species such as zooplankton often have a short generational time. Thus, density-mediated effects were expected to emerge even in short-term experiments. Furthermore, researchers sometimes manipulated the density of transmitter artificially to separate the density- and trait-mediated effects. When manipulating the density at once, cumulative response of density-mediated effect is unlikely to occur. Another reason for the lack of time-scale dependency is that the density-changes in transmitter are not always expected to accumulate. For example, in rocky intertidal systems (e.g., Menge, 1997), predator-prey interactions usually occur at the rocky patch level, and juveniles of transmitter species recruit into the patch in every spawning season. When recruitment of juveniles is limited by large spatial-scale processes such as climatic variations (e.g., Shanks & Roegner, 2007), cumulative responses over years may not emerge at the patch-level. Thus, the detection of cumulative indirect effects is dependent on the duration of the study conducted as well as the response characteristics of the transmitter species over time. I propose that researchers should pay attention to the time-scale dependency of indirect effects when transmitter species shows slow and cumulative responses, such as biomass change in long-lived plants.

The time-scale dependency of indirect effects means that the important process explaining system dynamics may be dependent on

time-scale of inducer population dynamics (Figure 5.1). When inducer species show relatively rapid population dynamics than the transmitter species, the indirect effect may act as a pulse perturbation, and immediate trait changes in transmitters will have considerable impacts on the responder species. When inducer dynamics is slow, however, press perturbation affects demography of transmitter species, and density-mediated effect may prevail. Actually, inducer populations can show both fast- and slow-time scale dynamics under transient conditions (Hastings, 2004). Under the transient conditions, which are not rare cases in nature, researchers should be in mind that the influential process can vary even in the interactions of the same species combinations.

Ecological role of large herbivores in terrestrial ecosystems can change over time, because various processes with different time scales may underlie the indirect effects of herbivore browsing. For example, effects of large herbivores on aboveground-belowground linkages are mediated by rapid and slow processes; browsed plant individuals show rapid changes in root and leaf litter quality, and species compositions gradually change to unpalatable plant dominated communities which provide low quality of litter (Bardgett & Wardle, 2003). The density of large herbivores occasionally shows a short-term fluctuation (e.g., Kaji et al., 2004; Valiex et al., 2008) as well as a gradual increase in long-term (e.g., Asada, 2011). When researchers examine the underlying mechanisms of ecological impacts by large herbivores under the fluctuating environments, researchers need to recognize the existence of time-scale dependency.
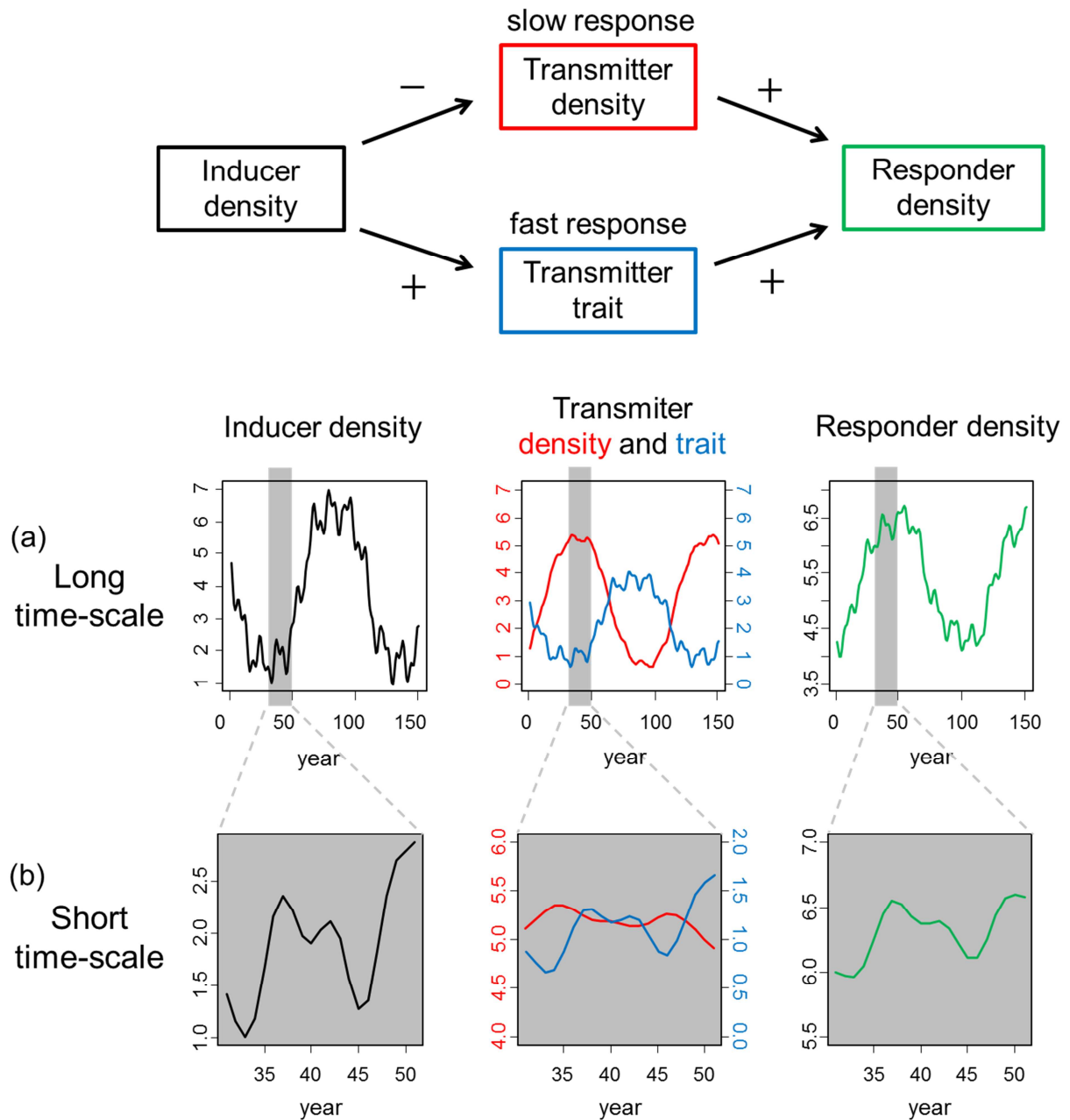
Figure 5.1.

The relationship between the time-scale of inducer dynamics and the relative strength of trait- and density-mediated indirect effects. (a) Inducer dynamics in long time-scale negatively affects responder density via cumulative density-mediated effect. (b) Inducer dynamics in short time-scale positively affects responder density via rapid trait-mediated effect.

Acknowledgment

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Appendix.

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