GfÖ Ecological Society of Germany, Austria and Switzerland
Basic and Applied Ecology 13 (2012) 260–267
Basic and Applied Ecology
www.elsevier.com/locate/baae

Variation in utilization of young leaves by a swallowtail butterfly across a deer density gradient

Shun Takagi*, Tadashi Miyashita

Laboratory of Biodiversity Science, School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Received 18 May 2011; accepted 8 March 2012

Abstract

Phytophagous insects can be affected by plant trait-mediated indirect effects of large herbivores, but little is known regarding how these effects change in response to different densities of large herbivores. To assess the response of an insect to plant qualitative change, the response of a woody vine (*Aristolochia kaempferi*) to browsing by sika deer (*Cervus nippon*) and utilization of young leaves by a swallowtail butterfly (*Byasa alcinous*) were investigated across a deer density gradient. Natural and simulated deer browsing stimulated the regrowth of *A. kaempferi* and improved nutritional and physical quality of leaves. Young leaves were frequently observed in areas with high deer densities. The proportion of young leaves among the leaves selected for oviposition was higher than their proportion of the total number of leaves. In areas with low deer densities, the utilization of young leaves by *B. alcinous* increased linearly with deer density, whereas in areas with high deer densities, the utilization of young leaves was around 90%.

Zusammenfassung

Phytophage Insekten können durch indirekte Effekte von Großherbivoren, die durch Pflanzen vermittelt werden, beeinflusst werden, aber wenig ist darüber bekannt, wie sich diese Effekte in Abhängigkeit von der Großherbivorendichte ändern.

Um die Antwort eines Insekts auf geänderte Pflanzenqualität zu bestimmen, untersuchten wir die Antwort einer holzigen Winde (*Aristolochia kaempferi*) auf den Fraß von Sikahirschen (*Cervus nippon*) sowie die Nutzung von jungen Blättern durch eine Schwalbenschwanzart (*Byasa alcinous*) entlang eines Gradienten unterschiedlicher Hirschdichte.

Natürliche und simulierte Hirschbeweidung stimulierte den Neuaustrieb von *A. kaempferi* und verbesserte die Nahrungsqualität der Blätter. Junge Blätter wurden häufig in Gebieten mit hohem Hirschbesatz gefunden. Der Anteil junger Blätter an den für die Eiablage ausgewählten Blättern war höher als ihr Anteil an der Zahl aller Blätter. In Gebieten mit geringem Hirschbesatz stieg die Nutzung der jungen Blätter durch *B. alcinous* linear mit der Hirschdichte an, während in Gebieten mit hohem Hirschbesatz die Nutzung der jungen Blätter bei etwa 90% lag.

Keywords: *Aristolochia kaempferi*; *Byasa alcinous*; *Cervus nippon*; Compensatory growth; Oviposition preference; Plant–herbivore interaction; Trait-mediated indirect interaction

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*Corresponding author. Tel.: +81 3 5841 7543; fax: +81 3 5841 8192.
E-mail addresses: takagi@es.a.u-tokyo.ac.jp, shun.takagi.1984@gmail.com (S. Takagi).

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doi:10.1016/j.baae.2012.03.003

Introduction

Large herbivores are one of the primary agents of disturbance in terrestrial ecosystems and have huge direct and indirect impacts on ecological communities (Rooney & Waller 2003; Côtè, Rooney, Tremblay, Dussault, & Waller 2004). Grazing or browsing by large herbivores not only reduces plant biomass but also changes plant architecture, chemical compounds, and nutritional quality for other herbivores (Nykänen & Koricheva 2004; Suominen & Danell 2006; Gómez & González-Megías 2007). In addition, plant quality is a key determinant of the fitness of phytophagous insects (Awmack & Leather 2002) and affects the abundance and community structure of insects on plants (Ohgushi 2005; Kaplan & Denno 2007).

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, Coleman, & Jones 2002; Utsumi, Ando, & Ohgushi 2009). In temperate areas, young leaves are generally unavailable to insects except during flush periods of their host plants, but compensatory growth of the plant induced by biotic and abiotic disturbances supplies young leaves aseasonally (Spiller & Agrawal 2003; Nakamura, Utsumi, Miki, & Ohgushi 2005; Utsumi et al. 2009). For example, browsing by large herbivores (a biotic disturbance) induces plant regrowth and secondary shoots, which often support a high abundance of phytophagous insects (e.g., Martinsen, Driebe, & Whitham 1998).

Differences in densities of insects and their eggs on browsed and unbrowsed plants have been documented (Martinsen et al. 1998; Olofsson & Strengbom 2000; Bailey & Whitham 2006). Such exclosure experiments in an area with a given large-herbivore density could not predict the "dose response" of insects to increasing large herbivore densities, when insects show non-linear responses that include a threshold or unimodal reaction and/or saturation (Suominen & Danell 2006). To resolve these problems, investigating the use and availability of suitable plant resources by insects in response to the density of large herbivores is necessary.

In this study, the effects of regrowth of a host plant (Aristolochiaceae: *Aristolochia kaempferi* Willd.) induced by sika deer (Cervidae: *Cervus nippon* Temminck) on utilization of young leaves by a swallowtail butterfly (Papilionidae: *Byasa alcinous* Klug) were examined in temperate forests in Japan. *A. kaempferi* is the only host plant of *B. alcinous* in our study area. Leaves of this plant usually mature in summer, but young soft 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 during the summer induces regrowth of *A. kaempferi*, young leaves are expected to be available. Leaf quality influences the performance of *B. alcinous*. Larvae that feed on mature tough leaves show low survival and prolonged larval periods (Takagi & Miyashita 2008). If adult females of *B. alcinous* selectively oviposit on young leaves, young leaves should be over-represented among leaves selected for oviposition compared to their proportion in the total leaf population. Furthermore, the proportion of young leaves selected by the butterfly (hereafter the "utilization of young leaves") is likely to reach a high level in areas with a high proportion of young leaves as a result of heavy deer browsing.

In this study the effect of deer browsing on the utilization of young leaves for oviposition by *B. alcinous* was examined across a deer-density gradient. Specifically, the following questions were addressed: (1) Does deer browsing stimulate the production of nutrient-rich leaves in *A. kaempferi*? (2) How does the frequency of young leaves of *A. kaempferi* change along a deer-density gradient? (3) Does *B. alcinous* selectively oviposit on young leaves of *A. kaempferi*? (4) How does the utilization of young leaves increase with deer density?

Materials and methods

Study system

Field surveys were conducted on the Boso peninsula (15 × 30 km; 35.12–26°N, 139.95–140.23°E; altitude 100–200 m), 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 km^2) 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 km^2 (Asada 2011). Deer density varies locally; 10–20 individuals/km^2 of deer have been established at the center of their distribution, and <10 individuals/km^2 of deer inhabit the peripheral areas. The density of the entire distribution of sika deer has been estimated at a resolution of 1 km^2 (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 & Nakama 1983), and the spatial gradient of pellet densities of the Boso sika deer population has been fairly stable since 2000 (Miyashita et al. 2008).

B. alcinous is a swallowtail butterfly found in eastern Asia. The life cycle of our study population is partially bivoltine (Kato 2001). Adults of the overwintering generation emerge in late April to early June; their offspring pupate and some individuals eclose in summer, but most of them diapause

Table 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 × 20 m^2 transect around each study site. Because leaf densities varied locally, minimum and maximum densities are shown.

Site name	Latitude/Longitude	Altitude (m)	Deer density (km^{-2})	Canopy openness (%)	Leaf density (10 m^{-2})	Site area (ha)
Fudago	35.20°N 140.14°E	151	14.8	12.8	0.0–5.5	1.5
Kagihara	35.18°N 140.04°E	187	9.4	18.1	7.0–55.5	0.25
Sasa	35.20°N 140.07°E	167	7.5	12.0	0.0–32.0	1.5
Takatsuka	35.26°N 140.23°E	123	4.5	13.7	4.5–42.5	0.75
Daifuku	35.25°N 140.15°E	164	3.4	14.9	4.5–101.5	0.75
Atago	35.12°N 139.95°E	204	2.4	11.2	1.5–33.5	0.75

at the pupal stage and eclose the next spring. Higher incidence of diapause under long day conditions is induced by low temperatures (Kato 2000) and low-quality leaves (Takagi & Miyashita 2008). Larval duration is 1–2 months in early summer. Old larvae often eat up all leaves of a host plant and move from plant to plant.

A. kaempferi, the host plant of *B. alcinous*, is 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 1).

Field experiments were conducted at Fudago, located in the 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 for 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, Kochi, Kobayashi, & Sirisampan 2005; Marenco, Antezana-Vera, & Nascimento 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 non-destructive measurement in an experimental cage (L × W × H = 7 × 2 × 3 m, covered with a 1-mm mesh net), which excluded deer browsing. Experimental plants of *A. kaempferi* collected near the cage were planted in May 2009. We collected average-sized plants which grew 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 *A. kaempferi* was analyzed by a general additive model. All statistical analyses in this study were performed with R 2.10.1 for Windows (R Development Core Team 2009).

Experiment 2. SPAD changes after natural deer browsing

Successive changes in SPAD values after deer browsing were measured outside of the deer exclosure. Sudden leaf disappearance occurred in four of the eight potted *A. kaempferi*, and three of these pots showed characteristics of mammalian browsing (probably caused by frequently occurring sika deer). SPAD values for three leaves of one potted plant could be monitored from regrowth to maturity because browsing occurred early in the experiment on this potted plant. This plant was browsed when the age of leaves was about 7 weeks. SPAD values for leaves of this plant were measured from 2 weeks before to 13.5 weeks after browsing. SPAD values at 2.5 weeks after browsing (immediately after resprouting) and those at 9.5 weeks after browsing (fully mature) were compared with those 1 week before browsing by student's *t*-test. The relationship between mean SPAD values at each measurement time and weeks after browsing was examined by single regression analysis.

Experiment 3. 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 4 potted *A. kaempferi*. In the experimental plots, 4 pots were covered by a 5 × 5-cm mesh 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 of deer exclosures were separately analyzed for each sampling date. 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 (GLMM), 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 deviance.

Experiment 4. 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. This clipping treatment was assumed to mean a disturbance similar in strength to natural deer browsing. C:N ratio and leaf toughness were measured to represent nutritional and physical quality, respectively. The force needed to pierce leaf tissue (an indicator of leaf toughness) was measured by penetrometer (RX-5; Aikoh Engineering, Osaka, Japan). 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.

Regional variation in the frequency of new leaves and their utilization by the butterfly

Frequency of young *A. kaempferi* leaves 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 1). At each site, leaves of *A. kaempferi* were chosen, SPAD values were measured, and the presence of *B. alcinous* eggs was recorded. Leaves were chosen from different shoots, and leaves smaller than 4 cm^2 were excluded from sampling to avoid bias towards young leaves. Leaves with eggs or first instar larvae were regarded as oviposited leaves because first instar larvae of *B. alcinous* are not highly mobile. Surveys of leaves continued until 10–20 leaves having eggs or larvae were counted. The total numbers of leaves examined ranged from 117 to 401 per 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 Results). To account for possible arbitrariness, different threshold values (±5) were used, but the results did not change significantly (data not shown).

To test whether the butterflies selectively oviposited on young leaves, randomization tests were performed. We randomly sampled SPAD values from the data of all the leaves observed in each site. Sample sizes were the same as those selected for oviposition in each site. Mean SPAD values were calculated from random selections 10 000 times and were compared to those of the leaves selected for oviposition. 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 also 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 link function and a binomial error structure. The effects of deer were tested by analysis of deviance.

Results

Plant response

The SPAD value of *A. kaempferi* increased with leaf age ($r^2 = 0.60$, $P < 0.001$; Fig. 1A). SPAD values showed a monotonic increase until about week 7, and thereafter the slope became lower. SPAD values reached approximately 35–45 in weeks 10–20. Plants considered to have suffered from deer browsing showed regrowth, and the SPAD value of the three leaves of regrowth increased with weeks after browsing ($F_{1,3} = 54.9$, $P = 0.005$; Fig. 1B). Leaves had lower values of SPAD shortly after browsing compared to leaves before browsing ($t_5 = 4.26$, $P = 0.008$); values recovered to the pre-browsing level 9.5 weeks after browsing ($t_5 = 0.260$, $P = 0.8$). Plants grown outside 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: $F_{1,3} = 41.7$, $P = 0.008$; Fig. 2). Number of leaves and SPAD values did not differ between inside and outside of the exclosures when the experiment started (number of leaves [mean ± 1 SE]:

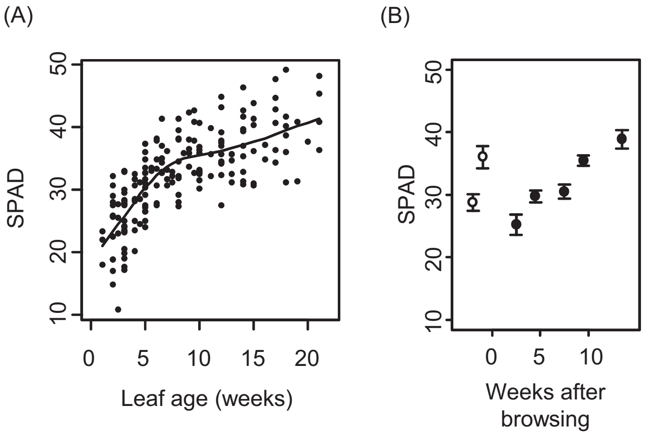

Fig. 1. (A) Relationship between SPAD value and age of *Aristolochia kaempferi* leaves in a deer-exclosed cage. The solid curve was estimated by general additive model. (B) Changes in SPAD values of *A. kaempferi* leaves after deer browsing. White and black circles are mean values before and after browsing, respectively. Error bars are ±1 SE.

inside [4.31 ± 0.373], outside [4.75 ± 0.395], $\chi^2_1 = 0.338$, $P = 0.560$; 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: $F_{1,3} = 26.1$, $P = 0.01$; Fig. 3).

Regional variation in the frequency of young leaves and their utilization by *B. alcinous*

Overall, the proportion of young leaves among the leaves selected for oviposition was higher than the proportion of young leaves in all the leaves observed. In 4 out of 6 sites, mean SPAD values were significantly lower than those from random selection (Table 2A). Because SPAD values of *A. kaempferi* reached a higher level than those in early season in 2 months with the level of about 35 (Fig. 1A), 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 ($\chi^2_1 = 6.12$, $P = 0.013$; $Y = 1/[1 + \exp(2.3 - 0.25 \times \text{deer density})]$; Table 2B, Fig. 4). Utilization of young leaves by *B. alcinous* was greater in high deer-density areas than in low-density areas ($\chi^2_1 = 13.63$, $P < 0.001$; $Y = 1/[1 + \exp(2.1 - 0.45 \times \text{deer density})]$; Table 2B, Fig. 4). In areas with high deer densities, about 90% of *B. alcinous* eggs were observed on young leaves.

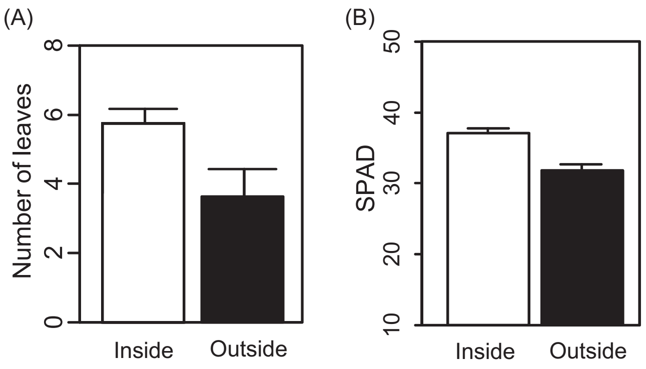

Fig. 2. Number of leaves per plant (A) and SPAD values (B) of *Aristolochia kaempferi* inside and outside of four deer exclosures in August (mean values + 1 SE are shown).

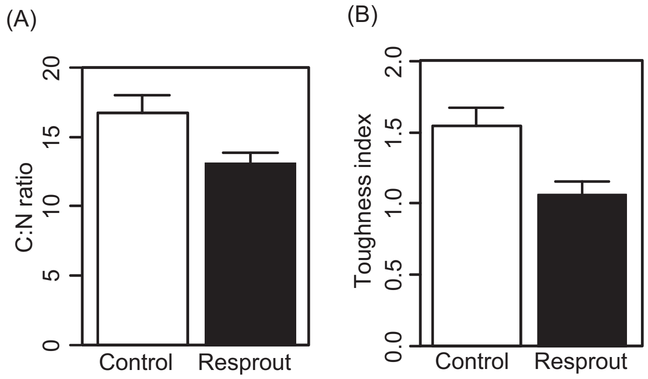

Fig. 3. CN ratio (A) and toughness (B) of *Aristolochia kaempferi* leaves for control and simulated browsing (resprout) treatments ($n = 4$ each, paired design; mean values + 1 SE are shown).

Discussion

Deer browsing stimulated the regrowth of *A. kaempferi*, which may compensate for aboveground biomass loss (Figs. 1–3). Simulated browsing also induced compensatory growth of *A. kaempferi* and improved nutritional and physical quality of leaves. Our previous research showed that *B. alcinous* larvae fed on tough and nutritionally poor leaves resulted in a 30% reduction in survival rate and elongation of the larval period compared to larvae fed on soft leaves (Takagi & Miyashita 2008). Because leaf quality experienced at the larval stage influences the fitness components of *B. alcinous*, improvement in leaf quality after herbivore browsing is likely to positively affect individual performance of *B. alcinous*. Positive effects of large herbivores on phytophagous insects via plant quality have been demonstrated previously (e.g., Martinsen et al. 1998; Bailey & Whitham 2006; but see Shimazaki & Miyashita 2002). One of the major mechanisms causing a positive indirect effect is compensatory growth of the plant (Haukioja, Ruohomäki, Senn, Suomela, & Walls 1990). Sprouting ability of plants is known to evolve under environments subject to frequent disturbances (Bond & Midgley 2003), and *A. kaempferi* can also 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.

Table 2. (A) SPAD values of the leaves selected for oviposition and all the leaves observed. Mean ± SE (upper row), minimum and maximum values (lower row) are shown. Mean SPAD values significantly lower ($P < 0.05$) than those from random selection are in bold. (B) The number of young and total leaves selected for oviposition, and of all leaves observed.

	Site name					
	Fudago	Kagihara	Sasa	Takatsuka	Daifuku	Atago
(A) SPAD						
Oviposited	**25.5 ± 1.50**	26.9 ± 1.35	**28.9 ± 2.35**	**33.1 ± 2.14**	**36.2 ± 2.50**	42.0 ± 1.64
	18.9–35.0	17.2–35.2	15.1–49.6	14.4–48.3	20.2–51.0	32.7–50.6
All	32.9 ± 0.31	27.8 ± 0.75	34.8 ± 0.33	37.2 ± 0.61	41.7 ± 0.38	43.7 ± 0.41
	18.9–44.9	10.4–43.1	15.1–51.6	14.4–55.4	19.4–63.9	23.4–54.4
(B) Young/Total						
Oviposited	9/10	11/12	11/16	11/20	7/15	1/11
All	190/318	90/117	200/401	80/235	40/305	12/177

Young leaves were frequently observed in areas with high deer densities (Fig. 4). 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 our study areas. Therefore, regional variation observed in this study was most likely caused by deer herbivory. However, some variation in the proportion of young leaves, especially among areas with high deer densities, was apparent, possibly as a result of local variation in per capita deer-browsing pressure. For example, local deer-browsing pressure is influenced not only by deer density but also by the surrounding landscape structures, such as agricultural fields (Takada, Asada, & Miyashita 2002).

As expected, the proportion of young leaves among the leaves selected for oviposition was higher than those in the total leaves (Fig. 4). Mean SPAD values were significantly lower than those from random selection except for two sites where the proportion of young leaves were too large or small (Table 2). These suggest that if both young and mature leaves were available, 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. High selectivity by insects could also be accomplished by a high detectability of resprouting plants owing to shoot elongation, even without oviposition preference. For example, some plant species produce vigorous and prolonged shoots after herbivory (Roininen, Price, & Bryant 1997; Schwenk & Strong 2011). In *A. kaempferi*, which grows on the forest floor, however, length of aboveground shoots with young leaves of regrowth plants was similar or even shorter than that of shoots with mature leaves of intact plants (unpublished data). Thus, selective oviposition on young leaves of *A. kaempferi* by *B. alcinous* appeared to be achieved by preference rather than detectability.

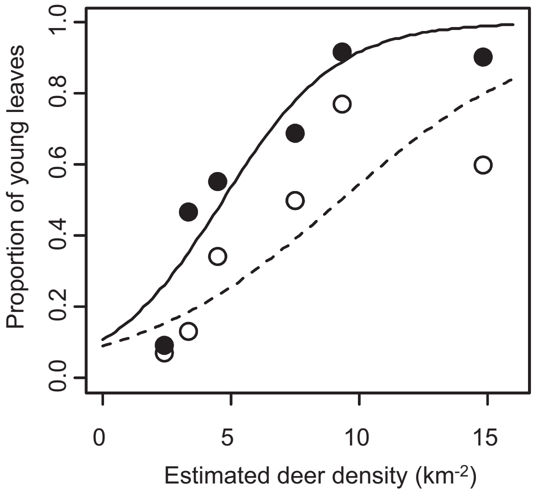

Fig. 4. Relationship between the proportion of young leaves in the total number of leaves observed (white circles, dashed curve) and the proportion of young leaves selected for oviposition by adult butterfly *Byasa alcinous* (black circles, solid curve). See also Table 2.

In accordance with the preference by *B. alcinous* for young leaves, most eggs were observed on young leaves in high deer-density areas, whereas they were found on mature leaves in the lowest deer-density area (Fig. 4). Young leaf recruitment after deer browsing significantly improved resource quality for *B. alcinous* at the regional scale. The frequency of young leaves among oviposited leaves was around 90% in areas with high deer density. This means that even if current deer densities increase further there, the opportunity to oviposit on young leaves 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.

Our study focused on a positive trait-mediated effect of deer on insects, but negative effects may also prevail under high deer-density conditions. Our experiment showed a decrease in the number of leaves under high browsing pressure (Fig. 2A). Although regional leaf densities of *A. kaempferi* did not show a conspicuous relationship to the current deer densities, leaf density was lowest in the area with the highest deer density (Table 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 negative effect is incidental omnivory on insects by large herbivores (Gómez & González-Megías 2007). However, as most of the deer browsing on *A. kaempferi* occurred in April to early May when *B. alcinous* is mainly in the pupal stage (unpublished data), incidental omnivory is unlikely to be important.

The experimental approach using exclosures is effective in clarifying the mechanism of indirect effects (Gómez & González-Megías 2007). However, as many of the earlier experiments were conducted in a single area with a given herbivore density, predicting the dose response of insects to deer density is impossible. To integrate the plant biomass- and quality-mediated effects of large herbivores on insect populations, it appears promising to conduct surveys across a density gradient of large herbivores on a large spatial scale, as reported here.

Acknowledgments

We thank S. Utsumi (the University of Tokyo) and two anonymous reviewers for helpful comments on an earlier version of this manuscript. We thank M. Asada (Chiba Biodiversity Center) for the information on the Boso deer population. We also thank the staff of the Tokyo University Forest in Chiba for supporting our fieldwork. This study was supported by a Grant-in-Aid for Japanese Society for Promotion of Science Fellows (21 5141) to S.T.

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