Aphid as a network creator for the plant-associated arthropod community and its consequence for plant reproductive success

Yoshino Ando*,1,2, Shunsuke Utsumi1,3 and Takayuki Ohgushi1

1Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113, Japan; 2Northern Forestry Research and Development Office, Field Science Center for Northern Biosphere, Hokkaido University, Nayoro, Hokkaido 096-0071, Japan; and 3Uryu Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Horokanai, Hokkaido 074-0741, Japan

Summary

1. Ecological communities consist of antagonistic and mutualistic interactions that can vary in their strength. Indirect effects act among species within or across trophic levels through multiple pathways in an interaction network. Although there are many studies showing indirect effects in ecological communities, we know little about how indirect effects impact the wider community by linking other direct and indirect interactions.

2. Herbivore-induced indirect effects are ubiquitous and powerful forces in structuring ecological communities. In a plant-associated network, aphids have the potential to connect multiple interactions through ant- and plant-mediated indirect effects on co-occurring and/or temporally separated species.

3. We examined how aphids affect the interaction network on tall goldenrod, Solidago altissima, based on data of arthropod species on it with and without the aphid, Uroleucon nigrotuberculatum, using structural equation modelling (SEM) analysis.

4. The presence of aphids greatly changed the strength of several species interactions throughout the season. In the early season, aphids had negative indirect effects on leafhoppers and moth caterpillars through ants. On the other hand, aphid-induced leaf regrowth decreased scale insects but increased grasshoppers in the late season, when the aphid was no longer present. Moreover, the aphid-generated interactions increased seed production of tall goldenrods, due to indirect pathways through the leaf regrowth.

5. The aphid thus played a critical role as a network creator in determining the interaction network by generating ant- and plant-mediated indirect effects. In particular, the aphid-induced leaf regrowth contributed to interaction diversity (i.e. total number of positive/negative interactions and link density per each species) by connecting arthropod species in the early and late seasons.

6. We disentangled the complexity of direct and indirect pathways generated by the aphid (i.e. keystone herbivore), which largely determines community structure of associated arthropods and plant reproductive success. Our study combining ant- and plant-mediated indirect interactions mediated by the aphid provides a basis of better understanding of the underlying mechanisms of how the impact of the keystone herbivore can spread through plant-based insect networks via direct and indirect pathways.

Key-words: ant-mediated indirect effect, arthropod community, interaction network, keystone herbivore, network creator, plant-mediated indirect effect, seed production, structural equation modelling, tall goldenrod

Introduction

Ecological communities consist of antagonistic and/or mutualistic interactions that can vary in their strength. In particular, predation and herbivory have received much attention because of their strong impacts on food web structure (e.g. Paine 1980; Risch & Carroll 1982; Carpenter, Kitchell & Hodgson 1985; Huntly 1991; McInnes et al. 1992). Recently, it has become widely accepted that the
strength and direction of an interaction between two species are often altered by the density or traits of a third species (i.e. density- or trait-mediated indirect effects; Werner & Peacor 2003; Ohgushi 2005). Indirect effects are powerful forces structuring ecological communities because they can negatively and positively influence species within or across trophic levels and be transmitted through multiple pathways in an interaction network. Although there are many studies showing indirect effects in ecological communities, only a few studies have demonstrated how indirect effects impact the wider community by linking other direct and indirect interactions (Utsumi, Ando & Miki 2010). To understand how ecological communities are organized, it is necessary to examine how multiple interactions including positive/negative and direct/indirect interactions influence each other in a network of interactions.

A plant–arthropod system is suitable for investigating how different types of interspecific interactions influence each other, because a wide range of herbivores share a host plant, and their natural enemies also colonize the plant for foraging and nesting. These arthropods may affect each other via apparent competition, trophic cascades, mutualism, exploitative competition and trait-mediated indirect interactions (Ohgushi 2005; Ohgushi, Craig & Price 2007; Utsumi, Ando & Miki 2010; Ohgushi, Schmitz & Holt 2012). Although past studies have mainly focused on interactions between co-occurring species, plant-mediated indirect interactions through herbivore-induced plant phenotypes have the potential to link temporally and/or spatially separated herbivores (Ohgushi 2005, 2008; Ohgushi, Craig & Price 2007; Ando, Utsumi & Ohgushi 2011; Poelman & Dicke 2014). Thus, plant-mediated indirect effects can critically shape plant-associated arthropod communities by connecting species separated in time and space. However, little is known about how multiple positive/negative and direct/indirect interactions affect each other through plant-mediated indirect effects (but see Ohgushi 2008; Yoshimoto & Nishida 2008; Krishnan et al. 2015). The lack of extensive information hinders our understanding of how and to what extent the interaction network has ecological consequences for community structure and biodiversity. In addition, the plant-based arthropod community may affect plant reproductive success, because herbivory can positively or negatively affect seed production, depending on the feeding mode and species identity of herbivores (Meyer & Root 1993).

Aphids are widespread herbivorous insects on many terrestrial plants. Several studies on ant–aphid mutualisms have illustrated that the mutualism can cause a dramatic change in the structure of the associated community by altering interactions between host plants and other community members (Floate & Whitham 1994; Wimp & Whitham 2001, 2007), but they did not consider the role of aphids as a keystone herbivore (Hunter 1992). In this context, aphids can change various plant traits in terms of growth, soluble nitrogen and amino acid contents, secondary compound concentrations, and resource allocation to roots, shoots and seeds (Moran & Whitham 1990; Waltz & Whitham 1997; Petersen & Sandström 2001; Wimp & Whitham 2007), suggesting that aphids have the potential to create an interaction network in the arthropod community through ant- and/or plant-mediated indirect effects.

The aphid Uroleucon nigrotuberculatum Olive is one of the most abundant herbivores on the tall goldenrod, Solidago altissima, in Japan. Ando, Utsumi & Ohgushi (2011) documented a community-wide effect of aphids on abundances of co-occurring and temporally separated dominant herbivores. In addition, Ando & Ohgushi (2008) showed the aphid’s effects on the dominant herbivores throughout the season: honeydew scattered by the aphid on adjacent leaves attracted an ant, Formica japonica Motschulsky, which removed co-occurring leafhoppers and geometrid moth caterpillars. Also, the aphid infestation induced subsequent branching followed by new leaf production (hereafter called ‘leaf regrowth’) late in the season, which affected the abundances of scale insects and grasshoppers differently in the late season, when the aphid was no longer present. Moreover, the scale insect’s honeydew attracted F. japonica. Thus, the interaction network may be mainly mediated by two types of indirect effects of the aphid: ant- and plant-mediated indirect effects. However, it has not been unravelled how multiple direct and indirect effects initiated by the aphid primarily shape the interaction network in plant-associated arthropod communities in terms of interaction link structure and interaction strength (i.e. the role of the aphid as a network creator). Furthermore, it is not understood how the interaction network affects plant seed production.

The primary objective of this study was to highlight the significant role of the aphid as a network creator. To do this, we focused on the aphid impact on the dominant arthropods and newly analysed data obtained from the aphid exclusion experiment described in Ando, Utsumi & Ohgushi (2011). Using structural equation modelling, we assessed differences in interaction networks with and without aphids on tall goldenrods: (i) we analysed an aphid-generated interaction network of arthropods, (ii) we explored the interaction strength of positive/negative and direct/indirect pathways and (iii) we examined how the aphid-generated interaction network influences seed production of the tall goldenrod.

Materials and methods

TALL GOLDENROD AND ARTHROPODS

Tall goldenrod, Solidago altissima Linn. (Compositae), is a rhizomatous perennial herb that was introduced to Japan from North America approximately 100 years ago (Shimizu 2003). It has spread widely in Japan and become one of the most abundant weeds. It grows in open and disturbed areas and frequently invades abandoned agricultural fields.
Uroleucon nigrotuberculatum Olive (Homoptera: Aphididae) is an aphid that feeds exclusively on terminal shoots of S. altissima, and it was also introduced from North America in the early 1990s (Otake 1999). The aphid has become common and abundant in Japan. It emerges from overwintered eggs in early March and occurs until early August. In our experimental field, aphids occurred in the early season (mid-May to August) and disappeared in the late season (September to October).

Although various predators and parasitoids attacking the aphid have been recorded in North America (Moran 1986), the aphid had been almost free from natural enemies in Japan (Otake 1999). In fact, we only observed two native ladybirds, Coccinella septempunctata and Harmonia axyridis, and one native crab spider, Misumenops tricuspidatus, as predators, but their predation was negligible in the study area (Y. Ando, personal observation).

APHID EXCLUSION EXPERIMENT

This experiment was conducted at a common garden of the Center for Ecological Research, Kyoto University, in Otsu, Shiga Prefecture, central Japan. To investigate effects of aphid colonization on other herbivorous insects and predators, we used the field data in the aphid exclusion experiment conducted in 2001 (see Ando, Utsumi & Ohgushi 2011 for detailed experimental design). We used 20 potted plants for the aphid-free treatment and another 20 plants for the aphid-exposed treatment in the common garden. The aphid-exposed plants were subjected to aphid colonization freely. We checked all plants every day from mid-May to August, and removed aphids on the aphid-free plants with forceps when they were found. To confirm whether aphid colonization occurred in the aphid-exposed treatment, we counted aphids on plants once a week until the end of August. To examine how the aphid affected the abundance of other arthropods, we surveyed potted plants three times a week from mid-May to late October. Abundance of each arthropod species on the aphid-free and aphid-exposed plants was recorded. To examine seasonal changes in abundance and species composition of insect herbivores, the data obtained from multiple surveys for the number of each arthropod species on individual plants were averaged per day (hereafter called ‘mean abundance’) in the early season (aphid-present season: mid-May to August) and late season (aphid-absent season: September to October). In addition, to assess whether aphids can affect the reproductive success of tall goldenrods, seeds were collected from all of the experimental plants in late November when they were matured and counted in the laboratory. Then, they were kept in an unlighted refrigerator at 5 °C for 3 months. To compare seed viability between aphid-exposed and aphid-free plants, 20 seeds were randomly selected from all of the seeds collected from individual experimental plants and were placed on wet filter paper in Petri dishes in an environmental chamber at 20 °C, LD 14:10 on 4 March 2002. After 4 weeks, the germinated seeds with emerged radicles were counted.

APHID-CREATED INTERACTION NETWORK ON TALL GOLDENRODS

To examine how and to what extent the aphid generated a new interaction network, we compared the aphid-absent and aphid-present networks, using structural equation modelling (SEM). We applied a bootstrapping method in SEMs to estimate the coefficient and standard error for each path, using AMOS 5.0 in spss (Arbuckle 2003). Bootstrapping is a versatile nonparametric statistical approach for data analysis and is advocated as the best approach to examine direct and indirect effects in equation models when sample size is small to moderate (n = 20–80) (Efron & Tibshirani 1993). In this study, we used adequate sample size for the aphid absence/presence networks (n = 20).

As aphid effects on abundances of other arthropods may depend on the feeding guilds, such as leaf chewers and sap-feeders (Ando, Utsumi & Ohgushi 2011), we selected a dominant species of each feeding guild in the early and late seasons as a possible arthropod species involved in aphid-absent/aphid-present networks: a leafhopper, Nephrotettix cincticeps Uhler (Homoptera: Deltocephalidae, constituting 66% of sap-feeders), and a moth caterpillar, Ascotis selenaria cretacea Butler (Lepidoptera: Geometridae, constituting 78% of leaf chewers), and a scale insect, Parasaissetia nigra Nietner (Homoptera: Coccidae, constituting 79% of sap-feeders), in the late season. Also, as predators, we selected a dominant ant, Formica japonica Motschulsky (Hymenoptera: Formicidae), in the early season (constituting 98% of ants) and in the late season (constituting 91% of ants), hereafter called ‘early ant’ in the early season and ‘late ant’ in the late season. As the aphid infestation enhances leaf production late in the season by inducing leaf regrowth, we used the rate of leaf increase as a measure of leaf regrowth. The rate of leaf increase in the early and late season was calculated by dividing leaf numbers on July 20 (early season) and September 10 (late season) by the leaf number on May 12 (before aphid colonization), respectively.

We examined the relationships between pairwise combinations of mean abundances of arthropods and leaf regrowth in the early season (aphid-present season) and the late season (aphid-absent season) using Pearson’s correlation coefficients (see Table S1, Supporting Information). Data for arthropods and leaf regrowth were log(n +1)-transformed before the analysis. Following the correlations between mean abundances of aphids, other insects and leaf regrowth (see Table S1), together with detected interactions among aphids, other arthropods and plants in Ando & Ohgushi (2008), we constructed hypothesized models for the networks of aphid-exposed plants (n = 20) and aphid-free plants (n = 20), respectively (Fig. 1). The leaf regrowth in the early season was excluded because there was no significant correlation between aphids and leaf regrowth (see Table S1). The hypothesized model for the aphid-present network (Fig. 1b) consists of the following pathways: the aphid effects on the leafhopper and moth caterpillar through the removal behaviour of early ants in the early season, and the aphid effects on the grasshopper and scale insect through the removal behaviour of early ants in the early season and the aphid effects on the leafhopper and scale insect on leaf regrowth in the late season, and a direct path between the early and late ants. Moreover, we added an indirect effect of the scale insects on the grasshoppers through late ants following the correlations (see Table S1). Also, we constructed a hypothesized model for the aphid-absent network (Fig. 1a) by excluding aphid effects from the hypothesized model for the aphid-present network. Mean abundance of each arthropod species in the early and late seasons was used in the SEM analysis.

All path coefficients were estimated through 1000 bootstraps of the data set for each plant, using Shrout and Bolger’s technique (Shrout & Bolger 2002). We assessed the significance of direct effects (i.e. path coefficients) using a bootstrap procedure (maximum likelihood method, bias-corrected 95% confidential intervals). Our goal was to find the best model to describe important interactions, for aphid-absent/aphid-present networks, with employing a model pruning strategy. Alternative structural equation models were generated by modifying the hypothesized model using a backward stepwise elimination process in which non-significant paths (labels A–K in Fig. 1a, labels A–I in Fig. 1b) were sequentially removed from the hypothesized model (starting with the least significant relationship) and evaluated for their contribution to model fit with a chi-square lack-of-fit test until all
relationships within the model were significant (Grace 2006). Such alternative models were accepted as a good fit to the data if the chi-square lack-of-fit test was non-significant ($P > 0.05$). Paths were retained in subsequent models if the chi-square probability became significant when deleted (Grace 2006). Finally, the best model was selected using Akaike Information Criterion values (AIC), root mean square error of approximation (RMSEA) and expected cross-validation index (ECVI), which were compared across all accepted models (e.g. Kim & Holt 2012). The lowest AIC, RMSEA and ECVI scores indicate the best fit (Grace 2006).

After we selected the best model, the strength of indirect effects was calculated by multiplying the path coefficients along the individual paths mediated by associated variables. The strength of total effects was calculated by adding path coefficients of direct effects and the strength of indirect effects. Then, we assessed the significance of indirect and total effects using a bootstrap procedure (maximum likelihood method, with bias-corrected 95% confidential intervals). We also calculated link density for arthropod communities (i.e. number of significant links per species) in aphid-absent/present networks. We counted both direct links between arthropod species and indirect links between early and late season species mediated by leaf regrowth. As for ant-mediated indirect links (i.e. links between aphids and moth caterpillars and between aphids and leafhoppers), we counted number of direct links via ants. Plant-mediated indirect links had significant total effects of aphids in the early season on scale insects, grasshoppers and late ants in late seasons (see Table 1). We also performed ANOVA to analyse the significance of the effects of aphids on the abundance of other herbivores, late ants and leaf regrowth. Mean abundance of each arthropod species and the rate of leaf increase for leaf regrowth were log ($n + 1$)-transformed prior to the analyses.

**DIRECT AND INDIRECT EFFECTS OF APHIDS ON PLANT REPRODUCTIVE SUCCESS**

To examine how the aphid influences female reproductive success of tall goldenrods through seed production, we conducted an additional SEM analysis. As leaf regrowth increases seed production (Y. Ando, personal observation), the aphid may directly or indirectly affect seed production through leaf regrowth. We examined the relationship between leaf regrowth and seed production using Pearson’s correlation coefficients, with log ($n + 1$)-transformed data of the number of regrowth leaves and the number of seeds of individual plants ($n = 40$). The number of regrowth leaves was obtained by subtracting the number of leaves on May 12 (before aphid colonization) from that on September 10 (late season). Then, we constructed hypothesized models including seed production for the aphid-absent network ($n = 20$) and aphid-present network ($n = 20$) (Fig. S1), based on the aphid-absent and aphid-present models with all potential interactions (Fig. 1). Number of seeds of individual plants was used as the variable for seed production. Paths from each herbivore and leaf regrowth to seed production were included in the hypothesized models. After the best model was selected, we assessed the significance of indirect and total effects of arthropods on seed production in the absence and presence of aphids using a bootstrap procedure (maximum likelihood method, with bias-corrected 95% confidential intervals). We analysed the significance of the effect of aphids on seed production using ANOVA ($n = 40$). Data were log ($n + 1$)-transformed prior to the analysis. Also, we compared germination rate in the absence and presence of the aphid using ANOVA, with arcsine-square-root-transformed germination percentage ($n = 20$).

**Results**

**APHID-ABSENT AND -PRESENT NETWORKS ON TALL GOLDENRODS**

For the aphid-absent network, model 12, which was selected as the best model (see Table S2), contained only two significant pathways (Fig. 2a): one with a positive effect and one with a negative effect in the late season (see Table S3), which generated an indirect negative effect of scale insects on grasshoppers through late ants. For the aphid-present network, model 9, which was selected as the best model (see Table S2), contained eight significant
Table 1. Matrices for total effects of each arthropod and leaf regrowth in SEM for (a) aphid-absent network and (b) aphid-present networks in Figs 2 and 3

<table>
<thead>
<tr>
<th></th>
<th>Late ant</th>
<th>Grasshopper</th>
<th>Seed production</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moth caterpillar</td>
<td>–</td>
<td>–</td>
<td>–0.491</td>
</tr>
<tr>
<td>Leafhopper</td>
<td>–</td>
<td>–</td>
<td>0.729</td>
</tr>
<tr>
<td>Scale insect</td>
<td>0.915*</td>
<td>–0.817*</td>
<td>–0.146</td>
</tr>
<tr>
<td>Late ant</td>
<td>–</td>
<td>–0.893*</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Early ant</th>
<th>Moth caterpillar</th>
<th>Leafhopper</th>
<th>Leaf regrowth in late season</th>
<th>Scale insect</th>
<th>Late ant</th>
<th>Grasshopper</th>
<th>Seed production</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aphid</td>
<td>0.933*</td>
<td>–0.677</td>
<td>–0.882*</td>
<td>0.808</td>
<td>–0.411*</td>
<td>–0.458*</td>
<td>0.498*</td>
<td>0.659</td>
</tr>
<tr>
<td>Early ant</td>
<td>–</td>
<td>–0.726*</td>
<td>–0.823*</td>
<td>–0.420</td>
<td>0.213</td>
<td>–0.126</td>
<td>–0.220</td>
<td>–0.249</td>
</tr>
<tr>
<td>Moth caterpillar</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–0.108</td>
<td>0.055</td>
<td>0.031</td>
<td>–0.063</td>
<td>–0.061</td>
</tr>
<tr>
<td>Leafhopper</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.565</td>
<td>–0.287</td>
<td>–0.160</td>
<td>0.331</td>
<td>0.317</td>
</tr>
<tr>
<td>Leaf regrowth in late season</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–0.508</td>
<td>–0.283</td>
<td></td>
<td>0.586</td>
<td>0.561</td>
</tr>
<tr>
<td>Scale insect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–0.558*</td>
<td></td>
<td>0.211</td>
<td>–0.107</td>
</tr>
<tr>
<td>Late ant</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–0.108</td>
<td></td>
<td>–0.055</td>
<td>–</td>
</tr>
<tr>
<td>Grasshopper</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td>–0.509</td>
<td>–</td>
</tr>
</tbody>
</table>

The first column indicates the explanatory variables, and the first row indicates the response variables. The total effect was calculated by adding direct effects (i.e. standardized path coefficients) and the strength of indirect effects (i.e. multiplying standardized path coefficients of direct effects). As all paths in aphid-absent/present networks (Fig. 2) were retained in the model including seed production for aphid-absent/aphid-present networks (Fig. 3), total effects calculated using the same path coefficients did not differ between models with/without seed production. Bold numerals show values significant at P < 0.05. Asterisks indicate significant direct and plant-mediated indirect links between arthropods, which were used for the calculation of link density.

Pathways (Fig. 2b): five with positive effects and three with negative effects (see Table S3). Note that aphids had direct and/or indirect effects on all arthropods in both seasons (Table 1). The total effects of aphids on early ants, grasshoppers and leaf regrowth were positive, while those on moth caterpillars, leafhoppers, scale insects and late ants were negative (Table 1). Thus, the aphid clearly enriched the arthropod network structure by increasing positive significant paths from one to five and negative significant paths from one to three. As a result, the aphid increased link density by 149% [0.67 for three species and two significant links in the aphid-absent network (Table 1a) vs. 1.0 for seven species and seven significant links in the aphid-present network (Table 1b)].

** Aphid effects on co-occurring herbivores **

In the aphid-present network (Fig. 2b), through the early ants, the aphid significantly affected the moth caterpillar (strength of indirect effect = –0.677) and the leafhopper (strength of indirect effect = –0.823). We call these effects ‘ant-mediated indirect effect’ hereafter. On the other hand, there were no significant paths in the early season in the aphid-absent network (Fig. 2a; see Table S3). The aphid decreased abundance of leafhoppers and moth caterpillars by 43% and 58%, respectively (ANOVA: *F*$_{1,38}$ = 45.66, *P* < 0.001 for leafhoppers; *F*$_{1,38}$ = 74.40, *P* < 0.001 for moth caterpillars).

** Aphid effects on leaf regrowth **

The total aphid effect on leaf regrowth was 0.808 (Table 1b), which was obtained by adding the direct and indirect effects in the aphid-present network (Fig. 2b). We detected a significantly positive path from the aphid to leaf regrowth. Also, the aphid significantly affected leaf regrowth through the ant-mediated indirect effect on moth caterpillars and leafhoppers (strength of indirect effects = 0.073 for the moth caterpillar, –0.465 for the leafhopper). In contrast, no significant effects of herbivores on leaf regrowth were detected in the aphid-absent network (Fig. 2a; see Table S3). The aphid increased leaf regrowth by 436% (ANOVA: *F*$_{1,38}$ = 4836.8, *P* < 0.001).

** Aphid effects on temporally separated herbivores in the late season **

Our SEM revealed that the aphid affected temporally separated herbivores in the late season, when the aphid was no longer present. The total aphid effect was negative on the scale insect but positive on the grasshopper (Table 1b).

In the aphid-present network (Fig. 2b), the aphid negatively affected the scale insect through a negative path from the leaf regrowth to the scale insect (strength of total effect = –0.411; Table 1b). In contrast, the aphid had a significantly positive effect on the grasshopper through the leaf regrowth via a positive path from the leaf regrowth to
the grasshopper (strength of indirect effect = 0.560). Furthermore, aphids slightly increased grasshoppers through another pathway. As the scale insect had a significantly negative impact on the grasshopper through the late ant (hereafter called the ‘effect of the scale insect–ant interaction’), the reduced effect of this interaction resulted in a significantly positive aphid effect on the grasshopper in the late season (strength of indirect effect = 0.025). On the other hand, in the aphid-absent network (Fig. 2a), there was only a significantly negative effect of the scale insect–ant interaction on grasshoppers (strength of indirect effect = −0.817; Table 1a). The aphid decreased scale insects by 41% but increased grasshoppers by 323% (ANOVA: $F_{1,38} = 126.36$, $P < 0.001$ for scale insects; $F_{1,38} = 52.38$, $P < 0.001$ for grasshoppers). Also, the aphid decreased late ants by 63% ($F_{1,38} = 49.07$, $P < 0.001$).

**APHID EFFECTS ON SEED PRODUCTION**

There was a significantly positive correlation between leaf regrowth and seed production ($r = 0.84$, $P < 0.001$). The best model 15 in the aphid-absent network (see Table S4) had three significant, direct paths (from the moth caterpillar, leafhopper and scale insects) to seed production (Fig. 3a; see Table S3). On the other hand, the best model 13 in the aphid-present network (see Table S4) had three significant, direct paths (from the aphid, leaf regrowth and grasshopper) to seed production (Fig. 3b; see Table S3).

In the best model for the aphid-present network (Fig. 3b), we detected three significant, indirect pathways from the aphid to the seed production (see Table S5). Through these pathways, the aphid had one positive and two negative effects on seed production (see Table S5). The strongest positive indirect effect of the aphid on seed production occurred through leaf regrowth alone (i.e. No. 2 in Table S5) (strength of indirect effect = 1.031). On the other hand, the negative indirect effects of the aphid on the seed production occurred through a pathway via ‘leaf regrowth–scale insect–late ant–grasshopper’ and a pathway via ‘leaf regrowth–grasshopper’ (i.e. No. 6 and 12 in Table S5, respectively) (total strength of indirect effects = −0.442). As a result, the total aphid effect on the seed production was positive (strength of total effect = 0.659; Table 1b), and aphids increased seed production by 138% (ANOVA: $F_{1,38} = 88.50$, $P < 0.001$). On the other hand, the germination rates were not significantly different between aphid-exposed and aphid-free plants (ANOVA: $F_{1,38} = 0.75$, $P = 0.392$; mean germination rates: 87% for aphid-free plants, 85% for aphid-exposed plants).

**Discussion**

We clearly showed that the aphid substantially increased interaction diversity (i.e. numbers of positive/negative interactions and link density) by connecting insect species in the early season and late season. Also, our study revealed the strong linkage of plant reproductive success and associated network structure: the aphid-generated interaction network improved the reproductive success of tall goldenrods by increasing seed production.

**APHID AS A NETWORK CREATORE**

The structure of the interaction network on tall goldenrods was surprisingly enriched in the presence of the aphid.
In contrast to two significant pathways in the absence of the aphid, 12 pathways (i.e. eight significant and four non-significant pathways) were detected in the presence of the aphid. The aphid altered the strength of many species interactions, thereby increasing the number of interactions. These interactions were both direct and indirect, and both negative and positive. Our results imply that the aphid plays a critical role as a network creator, with greatly increasing numbers of interactions and thus link density. Community ecologists have long recognized that one species can often influence many species within an overall community (i.e. keystone species; see Paine 1969, 1995; Power et al. 1996). Previous studies have focused on the role of keystone predators, which typically suppress many potential interspecific interactions (Paine 1969, 1995; Estes, Smith & Palmisano 1978; Risch & Carroll 1982; Beschta & Ripple 2015). Previous studies have focused on the role of keystone predators, which typically suppress many potential interspecific interactions (Paine 1969, 1995; Estes, Smith & Palmisano 1978; Risch & Carroll 1982; Beschta & Ripple 2015). In contrast, this study, for the first time, demonstrates that one herbivore species can drastically increase interaction diversity, suggesting an important role of keystone herbivores as network creators. It should also be noted that this function is generated by not only concurrent interactions but also temporally separated indirect interactions mediated by plant traits.

**HOW DOES THE APHID CREATE THE INTERACTION NETWORK ON TALL GOLDENRODS?**

This study revealed that an increase in interaction diversity was mainly mediated by (i) aphid-induced plant regrowth, (ii) hemipteran insect–ant interactions and (iii) connection between early- and late-season herbivores through indirect effects of the aphid.

**Via aphid-induced plant regrowth**

Several studies reported that attack by sap-feeders induces plant regrowth (Vranjic & Gullan 1990; Meyer & Whitlow 1992; Sudderth, Stinson & Bazzaz 2005). Our previous...
study documented aphid-induced plant regrowth of tall goldenrod (Ando & Ohgushi 2008). In the present study, not only a direct effect but also indirect effects of aphids stimulated leaf regrowth. Although not all of the aphid effects enhanced leaf regrowth, the overall aphid impact was positive because the positive direct effect of aphids overrode the negative indirect effect of aphids through the decrease in leafhopper numbers by the ant-mediated indirect effect (Fig. 2b). In fact, aphids increased leaf regrowth fourfold compared to that in the aphid-free plants.

Interestingly, in the presence of the aphid, a positive path from leafhoppers to leaf regrowth was detected, but it was diminished in the absence of the aphid (Fig. 2). This suggests that leafhoppers may enhance leaf regrowth induced by aphid attack, but may not induce regrowth by their own feeding alone. Thus, effects of other herbivores on leaf regrowth can be variable, depending on the presence or absence of the aphid.

**Via hemipteran-ant interactions**

Previous studies on community-wide impacts of aphids have mainly focused on how ant–aphid mutualism affects the abundance of co-occurring arthropods (e.g. Kaplan & Eubanks 2005; Eubanks & Styrsky 2006). Aphids decreased other herbivorous insects and predators through the removal by tending ants (Wimp & Whitham 2001). Such an ant-mediated indirect effect of aphids has been documented in both tight aphid–ant associations (i.e. ants intimately tend aphids) (Messina 1981; Wimp & Whitham 2001, 2007) and loose associations (i.e. ants only forage scattered aphid honeydew on leaves) as well as in our system (Del-Claro & Oliveira 1996, 1999, 2000; Stadler & Dixon 1999). Actually, aphids decreased the numbers of leafhoppers and moth caterpillars by 43% and 58%, respectively. As there were no other significant pathways between moth caterpillars, leafhoppers and aphids, the decrease in these herbivores was due to removal or predation by the ants.

Note that ants tending scale insects reduce insect herbivores (Bach 1991; Styrsky & Eubanks 2007). This was the case in our study as well: we detected negative, indirect effects of the scale insect–ant interaction on grasshoppers in the absence and presence of aphids. Interestingly, our SEM revealed that the negative path from tending ants to grasshoppers was significant in the aphid-absent network but was not significant in the aphid-present network. Thus, the aphid would alter the effect of the scale insect–ant interaction on grasshoppers by attenuating negative effects of tending ants.

**Via connection between early- and late-season herbivores**

Also note that the aphid-induced leaf regrowth can connect the early-season community to the late-season community. In fact, leaf regrowth was induced not only directly by aphids but also indirectly by an aphid–ant interaction through early-season herbivores, which in turn affected the abundances of scale insects, late ants and grasshoppers in the late season (Fig. 2b).

The aphid indirectly decreased scale insects by 41% through leaf regrowth, although the underlying mechanisms remain unclear. Also, scale insect survival was lower on plants with regrowth induced by aphids than on aphid-free plants without regrowth (Ando & Ohgushi 2008). As a result, the decreased numbers of scale insects could contribute to the reduced numbers of tending ants (i.e. late ants). On the other hand, the total aphid effect on grasshoppers was positive, with grasshoppers on aphid-exposed plants increasing three times more than those on aphid-free plants. Because the increased leaf mass and nitrogen resulting from leaf regrowth increases the number of grasshoppers (Ando & Ohgushi 2008), aphids would increase the number of grasshoppers through regrowth leaves.

**Consequences of the aphid-generated interaction network for plant reproductive success**

The present study also clearly demonstrated how the aphid-generated interaction network enhanced female reproductive success of tall goldenrods, as a further feature of the aphid’s role. The strong positive effect of the aphid on seed production was achieved by two paths: a positive path from the leaf regrowth and a negative path from the grasshopper to seed production. Aphid feeding induced lateral shoot growth with enhanced leaf regrowth, thereby increasing flower stalks and thus seed production (Fig. 3). On the other hand, aphids indirectly increased the number of grasshoppers, which may enhance herbivory, thereby decreasing the number of seeds. The positive effect on seed production via leaf regrowth outweighed the negative effect of increased numbers of grasshoppers in the late season.

Interactions between honeydew-producing hemipterans and ants potentially play a role in indirect plant defense; removal of herbivorous arthropods by ants can result in decreased plant damage and/or increased plant growth and reproduction (Styrsky & Eubanks 2007; Zhang, Zhang & Ma 2012). To examine the consequences of hemipteran–ant interactions, most previous studies have focused on the ant effect using ant-exclusion experiments (Styrsky & Eubanks 2007), ignoring the effect of aphids alone. In this context, we revealed a positive effect of aphids on seed production due to the leaf regrowth alone, overwhelming the ant-mediated indirect effects (see Table S5). This suggests that outcomes of aphid–ant interaction effects on plant reproductive performance can be variable (Zhang, Zhang & Ma 2012), depending on the plant-mediated indirect effects of aphids.

On the other hand, the following points should be noted when evaluating aphid effects on plant reproductive
success: the tall goldenrod is a perennial herb and we examined seed production only in the first year. Also, the seed size/number trade-off (Venable 1992; Paul-Victor & Turnbull 2009) is important for the future plant performance. As the germination rate did not differ between the aphid-free and aphid-exposed plants, the aphid feeding did not deteriorate seed viability. For a full understanding of the aphid’s role in the plant reproductive success, additional studies on the above points will be critical. Importantly, the SEM estimation of network structure in plant-based arthropod communities can provide profound insights into direct and indirect effects of herbivory by diverse insects on plant reproduction.

In conclusion, our study revealed that (i) the aphid is a keystone herbivore creating the insect interaction network on the tall goldenrod through ant- and plant-mediated indirect effects and (ii) the aphid indirectly enhances plant reproductive success due to the induced leaf regrowth, despite its feeding. Note that plant-mediated indirect interactions play an important role in increasing interaction diversity by connecting temporally separated species. As aphids are very common on a wide variety of terrestrial plants, future research should pay more attention to aphid impacts by combining ant- and plant-mediated indirect interactions, to gain a more exact understanding of the linkage of different types of indirect effects in plant-based arthropod communities.

Acknowledgements
We thank T. P. Craig for valuable comments on the manuscript and A. Otake for identifying the aphid. This study was partly supported by the Ministry of Education, Culture, Sports, Science and Technology Grant-in-Aid for Scientific Research (B-2070010, B-25291102 and 16H04842) to T. O., and for the 21st Century COE Program (A14) and the Global COE Program (A06) to Kyoto University.

Data accessibility
All data used are present in the manuscript and its Supporting Information.

References


Received 1 August 2015; accepted 29 September 2016

Handling Editor: Arjen Biere

**Supporting Information**

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig. S1.** Hypothesized structural equation models including seed production.

**Table S1.** Correlation coefficients between pairwise combinations of arthropods and leaf regrowth.

**Table S2.** Model selection for aphid-absent and aphid-present networks in Fig. 1.

**Table S3.** SEM estimates of unstandardized direct interaction strengths.

**Table S4.** Model selection for aphid-absent/present networks including seed production.

**Table S5.** Aphid effects on seed production through 14 pathways in SEM.