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RESEARCH ARTICLE

Relatedness of Neighboring Plants Alters the Expression of Indirect Defense Traits in an Extrafloral Nectary-Bearing Plant

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Abstract Different degrees of genetic relatedness among plants living in close proximity create differences in resource allocation and change the expression of defense traits. I investigated the plasticity of defense traits in young *Mallotus japonicus* growing with half-siblings or non-siblings, and examined direct defense traits (trichome density, pellucid dot density, and total phenolic content) and indirect defense traits (number of extrafloral nectaries, volume of extrafloral nectar, and number of food bodies) on the leaves. The plants growing with non-siblings invested proportionally more in shoots at high root biomass. Most defense traits did not differ between the two conditions, but the volume of extrafloral nectar was significantly larger with non-siblings. Although trichome density was not correlated with total plant biomass, some relationships between defense and growth differed significantly between the conditions, indicating a trade-off between plant growth and the expression of each defense trait (representing defense allocation costs). The chemical defense traits (pellucid dot density, total phenolic compounds) were negatively correlated with total biomass in plants growing with half-siblings, but were significantly positively correlated with total biomass in plants growing with non-siblings. The indirect defense traits were significantly positively correlated with total biomass in plants growing with non-siblings. These results suggest that the relatedness of neighboring plants alters their defense strategy and defense allocation costs, and that the local genetic relatedness of plants may play an important role in plant–animal interactions.

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Introduction

Intraspecific competition is a primary interaction in natural populations, because it is a fundamental aspect of population dynamics, the evolution of dispersal strategies, life histories, and the maintenance of genetic variation (Cheplick 1992; Rankin et al. 2007). In intraspecific competition, kin selection theory suggests that the relatedness of interacting individuals is potentially a major factor that determines resource allocation. Some plants can recognize the relatedness of neighboring individuals, and alter their resource allocation patterns depending on whether their roots encounter siblings or non-siblings (reviewed in Chen et al. 2012). For example, when plants grow with siblings, they may restrict their root and shoot proliferation, and directly increase their inclusive fitness by allowing more resources to be invested in growth or reproduction. In addition, the restriction of root and shoot proliferation can indirectly increase fitness by reducing competitive effects, thereby promoting the fitness of both the individual and its neighboring kin (Kelly 1996; Holzapfel and Alpert 2003; Dudley and File 2007). In contrast, when plants grow with non-siblings, they compete with these neighbors by increasing root development (Dudley and File 2007; Murphy and Dudley 2009; Biedrzycki et al. 2010). Thus, different conditions in the genetic neighborhood can induce differences in resource allocation if plants show phenotypic plasticity to kinship, causing differences in competition.

These differences in resource allocation can also change the expression of defense traits. The compensatory continuum hypothesis predicts that plants growing under conditions of low competition should allocate more resources to defenses such as the induction of tolerance to herbivory (Karban et al. 1989; Maschinski and Whitham 1989) than plants under high competition, because the expression of anti-herbivore defense traits is more costly under competitive conditions (Siemens et al. 2003; Cipollini 2007, 2010). Many studies have supported this hypothesis (Cipollini and Bergelson 2001; Kurashige and Agrawal 2005). In contrast, the defense stress benefit hypothesis predicts that additional beneficial functions of defense traits will emerge under competition, such as allelopathy and associational defense (Inderjit and Del Moral 1997; Lankau and Strauss 2007), thereby reducing the costs of allocation to defense traits and promoting their expression under highly competitive conditions (Siemens et al. 2003; Cipollini 2007; Boege 2010). If the compensatory continuum hypothesis is supported, then plants growing with siblings should invest more in defenses than plants growing with non-siblings. Conversely, if the defense stress benefit hypothesis is supported, then plants growing with non-siblings should invest more in defenses than they should when growing with siblings.

Many plant species express multiple direct and indirect defense traits (Agrawal and Spiller 2004; Kobayashi et al. 2008; Olson et al. 2009; Yamawo et al. 2012a). Direct defenses include physical means of defense, such as trichomes, tough leaves, and the production of chemical substances such as alkaloids and tannins (Howe and Westley 1988; Walters 2011). Indirect defenses are biotic: plants may bear extrafloral nectaries (EFNs), food bodies (FBs; lipid-rich particles), and domatia to attract natural enemies of herbivores (Koptur 1992; Rico-Gray and Oliveira 2007). EFN-bearing species, which entrust their defense to symbiotic ants (Koptur 1992; Rico-Gray and Oliveira 2007), often show simultaneous expression of additional defensive traits such as trichome and chemical defense substance (Steward and Keeler 1988; Agrawal and Spiller 2004; Kobayashi et al. 2008; Rudgers et al. 2004; Young et al. 2010; Yamawo et al. 2012a). Plants can change multiple defense traits in response to both competition and abiotic factors such as the availability of light, water, and nutrients (Boege 2010; Yamawo et al. 2014). Elucidating the defense tactics of plants requires an investigation of the relationships between multiple defense traits and genetic conditions. Though Karban et al. (2013) has shown that inducible defenses are enhanced by volatile cues from siblings rather than strangers, no one has yet examined whether constitutive defenses respond to the relatedness of neighbours.

In the present study, I tested the hypothesis that plants change their defense traits and allocation costs in response to the relatedness of neighboring plants. First, I investigated the plasticity of multiple defense traits of *Mallotus japonicus* in relation to the relatedness of neighboring individuals. Second, I characterized the relationships between plant biomass and the expression of defense traits to determine whether genetic conditions altered the defense allocation costs. I discuss the effect of genetic conditions on the expression of defense traits.

Materials and Methods

Study Species

Mallotus japonicus (Euphorbiaceae) is a pioneer plant that grows in gaps and disturbed areas in temperate regions of eastern Asia. It produces physical, chemical, and biotic defenses against herbivores (Yamawo et al. 2012a, b). Trichomes, which are produced on leaf surfaces, function as a physical defense (Yamawo et al. 2012b). Pellucid dots, also on leaf surfaces, contain toxic metabolic substances or essential oils (Wittstock and Gershenzon 2002), and function as a chemical defense (Yamawo et al. 2012b). The plant also bears EFNs on its leaf edges and FBs on its leaf and stem surfaces, attracting ants, which remove herbivores from the plant (Yamawo et al. 2012a, b). Various factors such as soil nutrients, light intensity, and leaf age can affect the expression of resistance traits and the shoot to root ratio (Yamawo et al. 2012a, b). The species is a dioecious tree (Horikawa 1972), and is wind- and insect-pollinated (Yamasaki and Sakai 2013). The seeds fall mostly under the parent trees, but some are dispersed by birds (Sato and Sakai 2005). Because these seeds germinate simultaneously in response to gap signals (Washitani and Takenaka 1987; Shimoda et al. 1994), the combination of pollination and dispersal mechanisms means that young *M. japonicus* plants often grow beside both siblings and non-siblings.

Cultivation of Young Plants of *M. japonicus*

In September 2011, I collected 20 seeds of *M. japonicus* from each of 10 female trees (200 in total) that were 5–7 m tall and growing at the forest edge on Mt. Daimi (34°41′N, 133°55′E, 72 to 159 m a.s.l.) and Mt. Tatsu-no-kuti (34°70′N, 133°96′E, 10–230 m a.s.l.) in Okayama, western Japan. Each female sample tree was more than 550 m from the next nearest sample tree, and was growing within 10 m of different male trees. Seeds from each female tree may therefore have had different pollen parents, because the amount of airborne pollen of *M. japonicus* decreases rapidly with increasing distance from a male tree and the pollen rarely travels more than 100 m from the male tree (Yamasaki and Sakai 2013). For simplicity, I refer to seeds collected from the same mother plant as “half-siblings” and seeds collected from different mother plants as “non-siblings” following procedures described by Lepik et al. (2012). On 15-Nov-2012, I filled ten plastic containers (5 cm × 5 cm × 4 cm) with wet soil to a depth of 3 cm. I sowed the collected seeds at a depth of 1 cm with half-sibling seeds. I maintained the containers in a growth chamber at 35 °C under a 12L–12D photoperiod for 24 h, because *M. japonicus* seeds germinate after experiencing high temperatures (Washitani and Takenaka 1987). I then maintained the container at 25 °C under the same photoperiod for 30 days and watered the soil every other day.

On 15-Dec-2012, I selected seedlings that had grown to ca. 3 cm in height. To test the effect of plant relatedness, I planted paired seedlings (either 30 pairs of half-siblings or 30 random pairs of non-siblings) 2 cm apart in a plastic pot (5 cm × 5 cm × 10 cm) containing 70 % red soil and 30 % humus. At the start of the experiment, each shoot had both cotyledons and the first adult leaf. To assess the possibility of grouping bias, I examined each plant’s height and defense traits on the first leaf on the first day of the grouping: the numbers of EFNs and FBs, volume of extrafloral nectar (EF-nectar), and the densities of trichomes and pellucid dots. The plant height was measured to an accuracy of 1 mm using a ruler. All FBs on the plant were then removed using a fine brush. To measure the nectar volume secreted from EFNs, all leaves of cultivated plants were washed with distilled water to remove accumulated EF-nectar, and wiped softly with Kim-towels (Jujo Kimberly, Tokyo, Japan). After 24 h, the FBs newly produced on each plant were counted. Then the nectar secreted within 24 h was collected from all EFNs in a 0.5-μL microcapillary tube (Drummond Scientific Co., Broomall, PA, USA). The EFNs on the first leaf were counted. The densities of trichomes and pellucid dots were examined by selecting a 0.79-cm^2 area near the center of the leaf’s undersurface and photographing the area using a digital camera (Cyber-shot T10; Sony Corp., Tokyo, Japan). The trichomes and pellucid dots in this area were then counted, and used to calculate their densities (no. cm^{-2}) for each leaf.

The pots were maintained for 50 days at 25 °C under a 12L–12D photoperiod. Water was applied every other day. On 4-Feb-2013, FBs were counted and EF-nectar was collected and analyzed as above.

Thereafter, all plants were collected and sandwiched in moisture-absorbing paper for 7 days. They were then divided into shoot and roots and dried at 30 °C for 10 days. These two parts of each plant were weighed separately to a precision of 0.1 mg on an electronic balance (BP211D; Sartorius, Goettingen, Germany). The fifth and greater fully expanded leaves from the apex of the plants were used to measure the expression of defense traits in the leaves, to exclude the effects of leaf age on this expression. The densities of trichomes and pellucid dots on the fifth leaf were examined as follows. Two small areas (each 23.7 mm^2) near both sides of the midrib on the leaf’s undersurface were selected at approximately one-fifth of the distance from the tip of the leaf toward the base. The trichomes and pellucid dots in each area were counted under a microscope (at 40×). The densities (no. cm^{-2}) were then calculated.

Finally, I measured the leaf contents of phenolic compounds, which provide a defense against many herbivorous arthropods (Feeny 1970; Dudt and Shure 1994). I powdered the dried fifth leaf of each plant in a mill, and extracted the total phenolics in 20 mg of leaf powder in 50 % methanol (10 mL) for 1 h in an ultrasonicated bath at 40 °C. The phenolic concentration (mg g^{-1}) was then measured by the Folin–Ciocalteu method (Julkunen-Tiitto 1985).

Statistical Analyses

Statistical analyses were performed using version 2.15.1 of the R software (R Development Core Team 2012). Plant heights and defense traits (number of trichomes, pellucid dot density, number of EFNs, volume of EF- nectar, and number of FBs) of the plants before the experiment were compared between the half-sibling and non-sibling conditions using generalized linear mixed-effects models (GLMM) with a Gaussian distribution and identity link, including the genotype (parent plant ID) as a random effect.

I measured phenotypic plasticity to half-sibling versus non-sibling conditions in biomass and other functional traits commonly associated with competition intensity, which are commonly used for this purpose (Weiner and Thomas 1992; Lin et al. 2014). Shoot, root, and total biomass of the plants were also compared between the half-sibling and non-sibling conditions using GLMMs with a Gaussian distribution and identity link, including the genotype (parent plant ID) as a random effect. To investigate the difference in allometry between the shoot and root biomass between the two conditions, I analyzed these relationships using GLMMs with a Gaussian distribution and identity link, including the genotype as a random effect. To examine the significance of the explanatory variables, I conducted a likelihood-ratio test in the GLMM analyses.

The indirect defense traits (number of EFNs, volume of EF-nectar, and number of FBs) and the direct defense traits (number of trichomes, density of pellucid dots, total phenolic compounds) after the experiment began were analyzed using principal-components analysis (PCA) after standardization. I used the *t* test to identify significant effects of genetic condition on the values of the first two principal components (PCs), and least-squares regression to analyze the relationships between the PCs and the defense traits.

Covariance matrices for the growth and defense traits between half-siblings and non-siblings were compared using Box's *M*-test. Correlations between total biomass and each defense trait in each genetic neighborhood condition were analyzed using least-squares regression.

Results

Plasticity of Growth and Defense Traits

Plant heights did not differ significantly between the two groups ($\beta \pm \mathrm{SE} = 3.03 \pm 0.03$, $\chi^2 = 0.408$, $P = 0.523$); both were 3.0 ± 0.1 cm (mean ± SD). No grouping bias was detected between the half-sibling and non-sibling conditions ($n = 30$ each) for any leaf characteristic examined at the start of the experiment: number of EFNs, $\beta \pm \mathrm{SE} = 0.10 \pm 0.12$, $\chi^2 = 0.311$, $P = 0.561$; number of FBs, $\beta \pm \mathrm{SE} = 0.50 \pm 0.31$, $\chi^2 = 0.303$, $P = 0.582$; volume of EF-nectar, $\beta \pm \mathrm{SE} = 0.10 \pm 0.01$, $\chi^2 = 0.352$, $P = 0.553$; trichome density, $\beta \pm \mathrm{SE} = 0.57 \pm 0.36$, $\chi^2 = 0.089$, $P = 0.765$; and pellucid dot density, $\beta \pm \mathrm{SE} = 29.03 \pm 4.76$, $\chi^2 = 1.489$, $P = 0.222$.

After 50 days of culture, the following parameters (mean ± SD, $df = 1$) did not differ significantly between the half-sibling and non-sibling conditions: shoot biomass (half-siblings, 0.29 ± 0.06 g; non-siblings, 0.32 ± 0.11 g; $\beta \pm \mathrm{SE} = 0.31 \pm 0.02$, $\chi^2 = 0.128$, $P = 0.720$), root biomass (half-siblings, 0.16 ± 0.06 g; non-siblings, 0.18 ± 0.06 g; $\beta \pm \mathrm{SE} = 0.18 \pm 0.01$, $\chi^2 = 0.613$, $P = 0.434$), and total biomass (half-siblings, 0.45 ± 0.11 g; non-siblings, 0.50 ± 0.16 g; $\beta \pm \mathrm{SE} = 0.49 \pm 0.03$, $\chi^2 = 0.270$, $P = 0.604$). However, the allometry between the shoot and root biomasses differed significantly between the half-sibling and non-sibling conditions ($\beta \pm \mathrm{SE} = 0.04 \pm 0.03$, $\chi^2 = 8.03$, $P = 0.005$): the plants growing with non-siblings invested less in shoot biomass at lower root biomass (<0.18 g) and more in shoot biomass at higher root biomass (Fig. 1).

The first two PCs from the PCA analysis of the indirect defense traits accounted for 99.9 % of the variance in the data. There was no effect of genetic condition on the PC1 scores (*t* test, $t = 1.49$, $P = 0.85$). However, there was a significant genetic condition effect on the PC2 scores (*t* test, $t = 4.19$, $P = 0.006$), which accounted for 41.5 % of the variance in the data. The scores of PC2 were significantly correlated with the volume of EF-nectar ($r = 0.97$, $P < 0.001$). The plants growing with non-siblings secreted larger volumes of EF-nectar than those growing with half-siblings.

The first two PCs from the PCA analysis of the direct defense traits accounted for 99.6 % of the variance in the data. There was no significant effect of genetic condition on the PC1 and PC2 scores (PC1, *t* test, $F = 1.21$, $P = 0.97$; PC2, *t*-test, $F = 2.72$, $P = 0.31$). Thus, the expression of direct defense traits did not differ between the half-siblings and the non-siblings.

Trade-offs Between Growth and Defense Traits Under the Two Genetic Conditions

The covariance matrices for the growth and defense traits differed significantly between the half-siblings and the non-siblings ($M = 1025.7$, $P < 0.0001$). The trichome density was not significantly correlated with plant biomass under either genetic condition (Table 1). In plants growing with half-siblings, the pellucid dot density and total phenolic compounds were significantly negatively correlated with total plant biomass, but in plants growing with non-siblings, they were significantly positively correlated with total biomass. In plants growing with half-siblings, the number of EFNs and FBs and the volume of EF-nectar

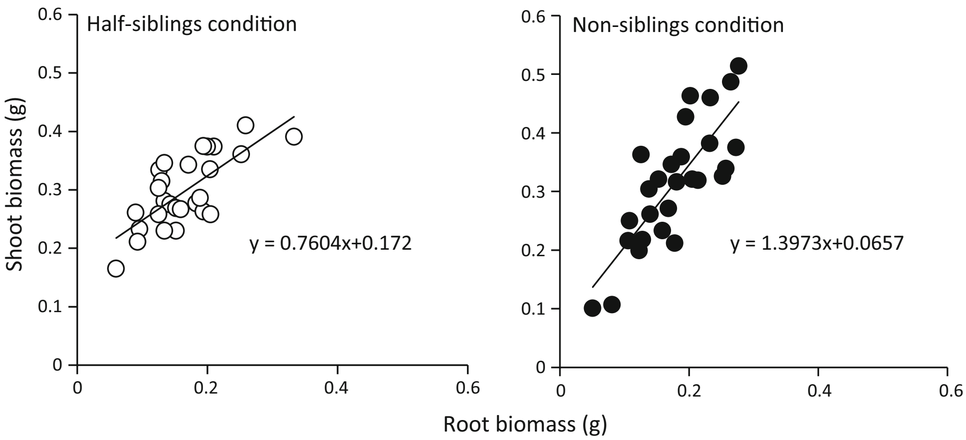

Fig. 1 The relationships between root and shoot biomass of *Mallotus japonicus* seedlings growing with half-siblings and non-siblings. The relationship differed significantly between the two genetic conditions (GLMM, $P < 0.01$)

Table 1 Relationships between total plant biomass and leaf defensive characteristics of *Mallotus japonicus* growing with half-siblings or non-siblings

Leaf characteristics	Half-siblings	Non-siblings
Trichome density	–0.199	0.142
Pellucid dot density	–0.376*	0.379*
Phenolic concentration	–0.483*	0.659**
No. of EFNs	0.185	0.403*
Volume of EF-nectar	0.125	0.399*
No. of FBs	0.056	0.632**

Significance levels: * $P < 0.05$, ** $P < 0.01$

Table 2 Defense traits of *Mallotus japonicus* seedlins growing with half-siblings or non-siblings

Leaf characteristics	Half-siblings	Non-siblings
Direct defense traits		
Trichome density (cm^{-1})	2.34 ± 1.37	3.00 ± 2.00
Pellucid dot density (cm^{-1})	26.22 ± 14.48	21.71 ± 8.43
Phenolic concentration ($mg\ g^{-1}$)	128.98 ± 30.21	114.23 ± 24.88
Indirect defense traits		
No. of EFNs	2.14 ± 0.52	2.14 ± 0.54
Volume of EF-nectar (ml)	0.04 ± 0.08	0.15 ± 0.19
No. of FBs	8.29 ± 14.06	7.79 ± 10.52

Mean ± SD

were not significantly correlated with total biomass, but in plants growing with non-siblings, they were significantly positively correlated with total biomass.

Discussion

Most earlier studies tested kin recognition in herbaceous plant species (reviewed in Chen et al. 2012). The plants growing with non-siblings invested more in shoot growth than plants growing with half-siblings at higher root biomass, but less in shoot growth at lower root biomass (Fig. 1). In general, pioneer plants often increase their allocation of photosynthate to shoots in response to competitors, because they face competition for light (Novoplansky 2009). *Mallotus japonicus* seedlings also increase their allocation to shoot growth under competition for light with plants of other species (Yamawo, unpublished data). In plants growing with non-siblings, *M. japonicus* seedlings may experience more intense competition, and change their resource allocation patterns accordingly. These results strongly suggest the existence of kin recognition in *M. japonicus*. The seedlings of this species often grow in tree-fall gaps or at forest edges (Washitani and Takenaka 1987; Shimoda et al. 1994), beside half-siblings or non-siblings. Under these conditions, it would be beneficial for *M. japonicus* seedlings to have the ability to recognize siblings.

How do *M. japonicus* plants recognize genetically related individuals? Research in other species indicates that plants can recognize the relatedness of nearby individuals through chemical cues received by their aboveground tissues (Karban et al. 2013), their belowground tissues (Biedrzycki et al. 2010), and potentially mycorrhizal networks (File et al. 2012a, b). *Mallotus japonicus* plants might also recognize the relatedness of neighboring individuals through such chemical cues or through mycorrhizal networks, but additional studies must be conducted to test these possibilities and provide details of the kin recognition mechanisms of these plants.

A few previous studies demonstrated that herbaceous plants increase their production of chemical defenses as competition increases (e.g., Barton and Bowers 2006; Boege 2010). In the present study, most defense traits, including the chemical defense traits, did not differ significantly between the genetic conditions (Table 2). These results did not support my hypothesis. The effects of competition on the expression of defense traits may differ among plant species or between life forms such as herbs and trees that take different times to mature. Thus, it is also possible that the duration of the experiment was too short to reveal differences in the expression of these defense traits in a tree species. My experiment was conducted for only 50 days, which is similar to the period used in a previous experiment with herbaceous plants (Barton and Bowers 2006), but trees take much longer to mature. In the future, a longer study should be conducted to reveal the long-term effects of genetic conditions on the expression of defense traits in trees.

However, despite the short study duration, the cost of resource allocation to chemical defense traits differed dramatically between plants growing with half-siblings and those with non-siblings. When growing with half-siblings, an increasing investment in pellucid dot production and in total phenolic content significantly reduced plant growth (Table 1). Many previous reports have described how the production of defensive chemical substances reduces plant growth and reproduction (e.g., Redman et al. 2001; Strauss et al. 2002). Consequently, these results suggest that the production of pellucid dots and phenolic compounds is costly for plants that are growing with half-siblings, which therefore decrease their allocation to these defenses. In contrast, in plants growing with non-siblings, the production of pellucid dots and of phenolic compounds was positively correlated with total biomass (Table 1). These results suggest that the use of chemical defense traits such as pellucid dots and phenolic compounds responds to the plant's competitive conditions, and that individuals that express high levels of chemical defenses may have an advantage when they are competing with non-siblings. Although the pellucid dot contents in *M. japonicus* have not been identified, the pellucid dots of an allied species, *Macaranga tanarius*, are known to secrete the prenylated flavanone nymphaeol-C (Guhling et al. 2005). Some studies in *M. tanarius* found that phenolic compounds, including nymphaeol-C, have allelopathic functions against other plants (Heil et al. 2002; Tseng et al. 2003). Phenolic compounds therefore seem to fulfill two defensive functions: anti-herbivore defense and allelopathy against competitive plants (Heil et al. 2002; Zhao-Hui et al. 2010). Because plants growing with non-siblings face higher competition than plants growing with half-siblings, a greater investment in chemical defenses may offer advantages. A similar result was reported in *Plantago* species in response to interspecific competition (Barton and Bowers 2006), supporting the defense stress benefit hypothesis (Inderjit and Del Moral 1997; Lankau and Strauss 2007).

In addition, the plants secreted significantly more EF-nectar when they were growing with non-siblings. The expression of direct defenses such as trichomes and chemical substances is costly (e.g., Vickery and Vickery 1981; Redman et al. 2001; Strauss et al. 2002) compared with the expression of indirect defenses (O'Dowd 1979; Katayama and Suzuki 2011). For example, the cost of producing secondary compounds in *Thea* (=*Camellia*) *sinensis* accounted for 30 % of leaf dry weight (Vickery and Vickery 1981). In contrast, the cost of producing EFNs is ca. 1 % of the total energy invested in leaves in *Ochroma pyramidale* (O'Dowd 1979). The present results demonstrate that indirect defense traits such as the number of EFNs and FBs and the volume of EF-nectar did not create a significant tradeoff with plant growth in plants growing with non-siblings (Table 1). These findings support the results of previous studies. Previous research has shown that the production of a large amount of EF-nectar rapidly attracts mutualistic ants in the field (e.g., O'Dowd 1979; Koptur 1992; Ness 2003; Rico-Gray and Oliveira 2007). In *M. japonicus*, ants visited plants that had large amounts of EF-nectar more often than they visited plants with smaller amounts of EF-nectar (Yamawo et al. 2012a, b). Pioneer plants can reduce their investment in chemical defenses when ants tend them (Dyer et al. 2001), and this may promotes more rapid growth (Yamawo 2012). The use of such biotic defenses might therefore give the plants a competitive advantage when they are growing with non-siblings. This suggests that the relationship with neighboring plants governs the dependency on a defensive mutualism with ants. The plants that were growing with non-siblings invested more in total defenses than plants that were growing with half-siblings, which supports the defense stress benefit hypothesis (Inderjit and Del Moral 1997; Lankau and Strauss 2007).

Induced defenses that depend on the release of volatile compounds from damaged plants are also affected by the degree of relatedness between the plants that emit the volatiles and those that receive them. Karban et al. (2013) demonstrated that *Artemisia tridentata* responds differently to cues from kin and from non-relatives, making it less likely that emitters will aid strangers and making it more likely that receivers will respond to cues from relatives. More effective defense strategies can therefore be added to the growing list of favorable consequences of kin recognition for plants. Although I did not investigate induced defenses, I hypothesize that the relatedness of neighboring individuals would affect the expression of induced defenses by *M. japonicus*. Plants have evolved induced defenses to reduce their defense cost, since these defenses are less likely to be expressed when they are not needed (reviewed in Karban 2011). The present study demonstrated that chemical defense traits are costly for plants that are growing with half-siblings (Table 1). These results also suggest that induced chemical defenses will develop in plants that are growing with half-siblings.

The induction of indirect defenses through increased production of EF-nectar may also develop in plants growing with half-siblings. Some studies have demonstrated that constitutive and induced defenses are often negatively correlated (Karban and Myers 1989; Zangerl and Rutledge 1996; Morris et al. 2006). In addition to observing indirect defense through the secretion of EF-nectar, Holland et al. (2009) reported a negative correlation between constitutive and induced defenses. The induction of EF-nectar is may large in the plants that were growing with half-siblings, because these plants secreted only a significantly small volume of EF-nectar than in plants growing with non-siblings (Table 2).

In conclusion, this study demonstrated phenotypic plasticity of the indirect defense traits of *M. japonicus* in response to the degree of relatedness of neighboring plants. In addition, differences in the relatedness of neighboring plants altered the relationships between investments in growth and plant defense traits. Thus, these results support the my hypothesis that plants change their defense traits in response to the relatedness of neighboring plants. Although it has been previously recognized that the relatedness of competitors can alter the resistance phenotypes of plants, the role of kin recognition for trees has not been previously appreciated. My results suggest that the relatedness of the plants and their competitors could play an important role in plant–animal interactions and the evolution of plant defense traits, and therefore provide new information on the adaptive significance of kin recognition in woody plants. However, further studies must be conducted to ascertain the effect of genetic relatedness on the evolution of plant defense strategies in the field. In addition, the study should be extended to a longer period and investigation of the defense traits should be conducted at multiple points in time to account for the long maturation period of trees.

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