RESEARCH ARTICLE

Intraspecific neighbourhood effect: Population-level consequence of aggregation of highly defended plants

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Abstract

1. There is increasing evidence that herbivore–plant interactions on a focal plant species are influenced by interspecific neighbourhood effects via neighbouring plants (i.e. an associational effect). However, intraspecific neighborhood effects imposed by plant traits have been less appreciated. Specifically, the significance of intraspecific neighbourhood effects in population-level consequences of plants has been totally overlooked.

2. Using two varieties of *Nicotiana tabacum* (high- and low-nicotine), we evaluated the neighbourhood effects based on patch-level interactions in a split-plot 3 × 3 factorial experiment that manipulated number of plants (4, 9 and 16 plants) and culture type (monoculture plots with high- and low-nicotine plants, and polyculture plot) in an experimental garden.

3. We found that herbivore visits on plants varied depending on the number of plants per patch and culture type. Presence of more high-nicotine plants decreased herbivore visits in the four plant plots, and presence of high-nicotine plants in the nine plant plots decreased herbivore visits on both high- and low-nicotine plants. In contrast, in the 16 plant plots, herbivore visits on high-nicotine plants in polyculture plots were lower than others, including those on high-nicotine plants in monoculture plots.

4. Our findings clearly demonstrated that the intraspecific neighbourhood effect could occur depending on the aggregation of highly defended plants (i.e. high density and/or plant-spacing). This study suggests that multiple mechanisms for the neighbourhood effect simultaneously worked, depending on the patch size and composition of defensive traits of individual plants, and that intraspecific neighbourhood effects may influence population-level consequences for plant–herbivore interaction.

KEYWORDS

alkaloid, associational effect, defensive traits, density-dependency, herbivory, intraspecific neighbourhood effect, nicotine, plant–herbivore interaction

1 | INTRODUCTION

A wide range of plant–herbivore interactions are largely influenced by neighbouring plants (i.e. neighbourhood effects: reviewed by Barbosa et al., 2009; Underwood, Inouye, & Hambäck, 2014). The presence of specific neighbour species may increase or decrease attractiveness of other plant species to herbivores, which is referred to as associational susceptibility (Letourneau, 1995) and resistance (Tahvanainen & Root, 1972) respectively. Although there is an increasing evidence that heterospecific neighbours affect the likelihood of herbivory on focal individual plants (i.e. associational effect; Underwood et al., 2014), there has been less attention to conspecific neighbours (Agrawal, Lau, & Hambäck, 2006; Andow, 1991; Barbosa et al., 2009). In this context, more recent studies have argued that intraspecific variations in biological environments caused by local spatial distribution of plants and their phenotypic and/or genotypic variations can also induce neighbourhood effects (Coverdale, Goheen, Palmer, & Pringle, 2018; Ida et al., 2018; Koricheva & Hayes, 2018; Sato & Kudoh, 2015). For instance, plant genotypic diversity could influence visits of arthropods, including herbivores and predators, to host plants and alter associated arthropod communities (Crutsinger et al., 2006; Wetzel, Aflitto, & Thaler, 2018). While potential mechanisms of neighbourhood effects in local environments (e.g. repellent, decoy, dilution and/or attraction effects) have been elucidated, how intraspecific neighbourhood effects within a population determine spatial distribution of herbivores and population-level consequences for plant–herbivore interaction remain unclear.

To understand how neighbourhood effects in plant–herbivore interactions affect the herbivore distribution, it is essential to know how the neighbourhood effects on and herbivores' selection of the focal plant vary in response to the density of conspecific neighbours (or neighbouring plants with same phenotype and/or genotype) and the frequency (or relative density) of neighbouring heterospecific plants (or neighboring plants with different phenotype and/or genotype) at the patch level (see Kim & Underwood, 2015). This is because in nature individual plants of a single species are often clumped in distribution, and they have phenotypic and/or genotypic variations in traits such as defences (Agrawal, Conner, Johnson, & Wallsgrove, 2002; Baldwin, 1999; Goodger, Capon, & Woodrow, 2002; Simms & Rausher, 1987). Furthermore, neighbourhood effects caused by defensive chemical traits (e.g. VOCs and alkaloids) are effective only within a relatively short distance (e.g. Ida et al., 2018; Karban, Shiojiri, Huntzinger, & McCall, 2006). Therefore, neighbourhood effects on individual plants may vary within a population depending on the spatial arrangement of phenotypes, causing spatial variation in intraspecific neighbourhood effects. Previous studies showed that neighbourhood effects or associational effects are influenced by both density and frequency (i.e. relative density) effects of the local patch, but most of the studies have overlooked the nonlinearity of these effects (Hambäck, Inouye, Andersson, & Underwood, 2014; Kim & Underwood, 2015). Such nonlinearities predict that plant–herbivore interactions can vary depending on the number of

plants in a patch, resulting in diverse outcomes of neighbourhood effects within a population. Specifically, evaluation of density-dependent neighbourhood effects across a range of values is required to understand how intraspecific plant–plant interactions mediated by herbivory can contribute to population-level processes.

Variation in defensive traits among plants and how herbivores respond to the defence traits as attractant or repellent cues are also crucial factors affecting neighbourhood effects within a population (Augner, 1994). First, several mechanisms (e.g. attractant-decoy and repellent hypotheses; Atsatt & O'Dowd, 1976; Tahvanainen & Root, 1972) of neighbourhood effects work in plants with both high and low defences because these mechanisms depend on relative palatability among individual plants. Second, variations in defensive traits would result in various consequences for herbivores' plant selection. Plant defence against herbivores is often effective but it imposes energy costs associated with its production (Agrawal, 2000; Agrawal et al., 2002). As optimal defence theory predicts (Rhoades, 1979; Stamp, 2003), plants thus evolve defensive levels that are positively associated with herbivory level and negatively associated with allocation and/or ecological cost. Therefore, spatial heterogeneity in plant defence should be examined at the population level because intensity of herbivory often covaries with abiotic factors along an environmental gradient (such as season length and temperature correlated with longitude and/or altitude; Pellissier, Roger, Bilat, & Rasmann, 2014; Rasmann, Pellissier, Defossez, Jactel, & Kunstler, 2014). Such variations in defensive traits also can be found at the patch level. In fact, Augner (1994) theoretically argued that both defended and undefended plants may coexist if undefended plants can reduce the risk of herbivory by associating with defended plants. Furthermore, in our experimental study (Ida et al., 2018), an intraspecific neighbourhood effect caused by nicotine of *Nicotiana tabacum* plants could increase the level of defence against herbivores at the patch level. In fact, both highly and lowly defended plants that are associated with highly defended ones receive protection from herbivory through patch avoidance of grasshoppers (Ida et al., 2018). This result suggests that neighbourhood effects at the patch level are most likely to be influenced by the spatial distribution of plants with various defence levels, which include different trade-offs between investment in defence and growth/reproduction. Consequently, evaluating density-dependent neighbourhood effects in terms of patch-level interactions is the first step to understand the exact roles of neighbourhood effects at the population level. In this way, intraspecific neighbourhood effects can be integrated into our understanding of spatial distribution of herbivores mediated by defensive traits of plants.

To test the intraspecific neighbourhood effects, we used two varieties of tobacco, *Nicotiana tabacum* L., with different amount of nicotine (high- and low-nicotine plants) in a 3 × 3 factorial experiment manipulating number of plants per plot (4, 9 and 16 plants) and culture type (monoculture plots with high- and low-nicotine plants and polyculture plot) in an experimental garden. Our previous study showed that herbivore visits to plants growing with high-nicotine neighbours decreased in this species, and that such

positive neighbourhood effect occurred in plants with both high or low nicotine (Ida et al., 2018). However, the mechanisms underlying this neighbourhood effect at different densities of the *N. tabacum* varieties still remain unclear. These mechanisms may be tested with an additive design that holds focal plant number constant and manipulates neighbour number, or with a replacement design that manipulates plant composition (i.e. relative number of the focal and neighbour plants). These two experimental approaches tend to have different outcomes (Hambäck et al., 2014; Kim & Underwood, 2015). Hambäck et al. (2014) theoretically demonstrated that a neighbourhood effect is commonly observed in the replacement design (i.e. monoculture vs. polyculture plots within same number of plants per plot) because dilution effects (e.g. Hambäck et al., 2014; Otway, Hector, & Lawton, 2005) are generally weak when the number of plants per patch remains constant and when herbivore visits between the focal and neighbouring plants (i.e. selective grazing or attractant-decoy hypotheses; Atsatt & O'Dowd, 1976) shift depending on palatability of the plant. Our experimental design using varieties of *N. tabacum* with consideration of number of plants and culture type allowed us to test multiple mechanisms of the neighbourhood effect occurring simultaneously across a range of densities (three levels: 4, 9 and 16 plants) and frequencies (two types: monoculture plots [all plants in a plot were one of the varieties] or polyculture plots [plants in a plot were equally divided half in two varieties]). Furthermore, we examined variation in the magnitude of each neighbourhood effect, depending on the local plant–plant interactions, and integrate understanding of neighbourhood effects at population level. We specifically asked: (a) whether intraspecific neighbourhood effects in a plant–herbivore interaction vary depending on the number of plants per plot, (b) whether density-dependent neighbourhood effects vary between varieties with different nicotine levels and (c) whether the neighbourhood effects vary depending on the plant composition of the patch (i.e. monoculture or polyculture plots).

2 | MATERIALS AND METHODS

2.1 | Study site and species

This study was conducted in a 400-m^2 common garden at the Center for Ecological Research, Kyoto University in Otsu, Japan (34°58′N, 135°57′E) from February to August 2016. The study species was tobacco, *Nicotiana tabacum* L. (Solanaceae), an annual herbaceous plant that is often cultivated as a commercial product. The plant produces alkaloids (nicotine) that act as a defensive chemical against herbivores. We used two varieties of *N. tabacum*: Burley 21 (hereafter, high-nicotine variety) and LA burley 21 (hereafter, low-nicotine variety). High-nicotine plants had more than three times greater nicotine in leaves than low-nicotine plants (Ida et al., 2018). We grew seedlings of both *N. tabacum* varieties (provided by the Japan Tobacco Inc.) starting in February in greenhouses maintained at 25/20°C (day/night), and

all seedlings were watered daily. After the plants had four to six leaves, they were individually transplanted into pots (12 cm in diameter) filled with commercial soil and fertilizer and covered with fine-meshed spectrally neutral vinylon cloth (Unitika vinylon #520, Unitika) to exclude insect attacks; the cloth reduces light to 85%. All plants were watered as needed. The plants reached a maximum height of about 1.2 m.

2.2 | Experimental design

Just before elongation of flower stalks, 261 plants (130 high-nicotine variety and 131 low-nicotine variety) were randomly selected from the seedlings grown in the greenhouse. To determine the neighbourhood characteristics that affect herbivore visits, we conducted a split-plot 3 × 3 factorial experiment manipulating the plant density, number of plants per plot (three levels: 4, 9 and 16 plants), the plant frequency and culture type (three types: monoculture with high-nicotine variety, monoculture with low-nicotine variety and polyculture with high- and low-nicotine varieties). Our previous study using the same varieties of *N. tabacum* in the same garden demonstrated that the neighbourhood effect of high-nicotine plants drastically declined with distance and tended towards zero within 1.5–2 m (Ida et al., 2018). We therefore prepared twenty-seven 2 m × 2 m plots (i.e. 3 plant numbers × 3 culture types × 3 replications) with at least 1 m separation between the nearest plots within the site. The plants were transplanted into the prepared site at each number of plants, and assigned at equal intervals in a grid pattern within each 2 m × 2 m plot (each plant is 66 cm, 50 cm and 40 cm away from the nearest plant within the 4 plant, 9 plant and 16 plant plots respectively). Thus, plant number treatment varied simultaneously in number of plants and plant spacing within each 2 m × 2 m plot. In the polyculture plots, high- and low-nicotine plants alternated (see Figure 1). The polyculture plots with nine plants had two combinations: 5–4 and 4–5 plants of high- and low-nicotine plants respectively. In this study, we defined the number of plants (i.e. 4, 9 and 16) within the 2 m × 2 m plot as patch size. Hence, density of each variety in monoculture plots corresponded to patch size, while that in polyculture plots corresponded to half the number of plants per plot. The frequencies (i.e. relative densities) of varieties were 1 (monoculture plots) and 0.5 (polyculture plots). Two plants (one high-nicotine plant in a polyculture with 16 plants and one low-nicotine plant from a polyculture with 9 plants) were eliminated from the analyses because they wilted during the experiment.

To assess the plant–insect interactions in relation to number of plants and culture type, we surveyed herbivorous insects on all plants at almost 1-week interval between 28 May and 8 August (nine times in total). We focused on visits of grasshoppers (mostly *Atractomorpha lata*) as the herbivore attack in this study because other herbivores (e.g. lepidopteran caterpillars) were negligible in this common garden. In our preliminary observations, *N. tabacum* plants that received more visits by herbivores had greater damage on leaves and tended to decelerate the subsequent growth.
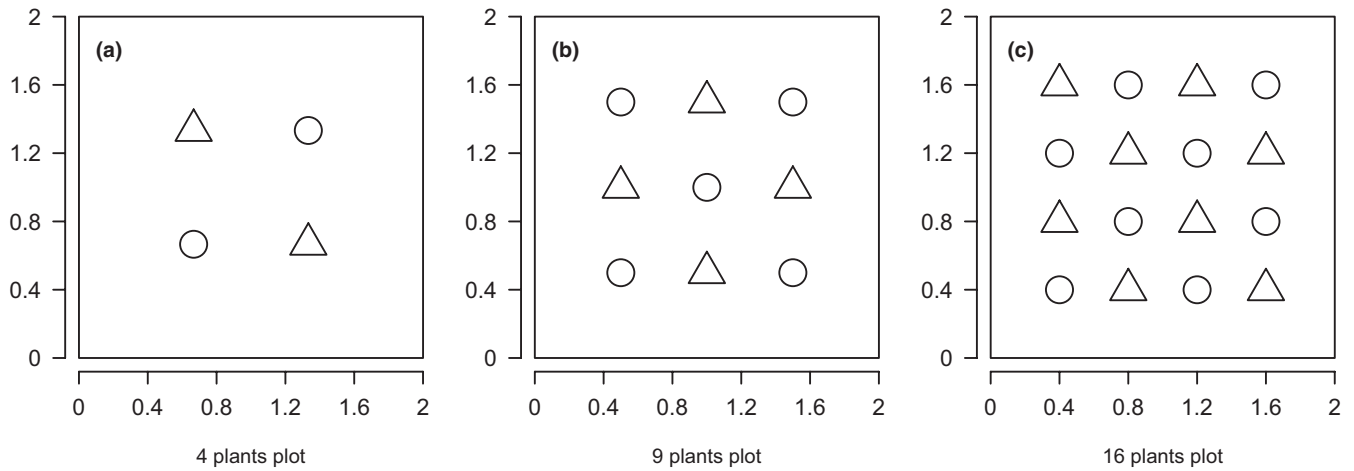
FIGURE 1 Experimental design of the effects of culture type on number of herbivores on individual *Nicotiana tabacum* plants. Plants within a 2 m × 2 m plot (a: 4 plants, b: 9 plants, and c: 16 plants) were randomly assigned to positions within plots. In monocultures, all plants were either high- or low-nicotine plants; positions indicated by open triangles and open circles were the same variety in these plots. In polycultures, high- and low-nicotine varieties alternated positions; in each polyculture plot, variety was randomly assigned to either open triangle or open circle positions. The analyses of herbivore visits were conducted from two perspectives. In the plot level analysis, total numbers of herbivore visits per plot were analysed. In the individual-plant level analysis, number of herbivore visits on individual plants was analysed
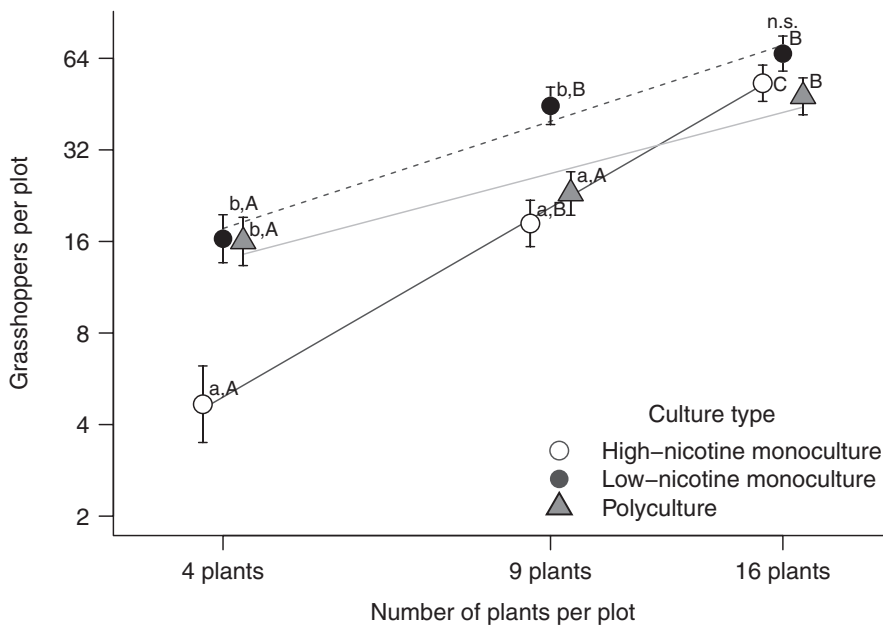

FIGURE 2 Effects of number of plants and culture type on least-squares mean (±*SE*) total number of grasshoppers on *Nicotiana tabacum* per plot (plot-level analysis). Least squares means with different small letters are significantly different within plots with same number of plants; those with different capital letters are significantly different among three levels of plant numbers within a culture type. Regression lines were obtained by analysing the number of grasshoppers considering logarithm number of plants per plot as continuous explanatory variable data using generalized linear model. Note the logarithmic scaling of the ordinate

2.3 | Data analysis

We analysed the effects of number of plants per plot and culture type on herbivore visits with generalized linear model (GLM; McCullagh & Nelder, 1989) or generalized linear mixed model (GLMM; Stroup, 2013) as implemented with the GLIMMIX procedure (*F* tests, with degrees of freedom calculated according to Kenward & Roger, 1997) of SAS version 9.4 (SAS Institute Inc., 2013). All analyses of grasshopper visits to the plants involved negative binomial distributions and ln-link functions. Because number of grasshoppers per census day was not large enough, we analysed the pooled number of grasshoppers across all sampling times. Analyses of grasshopper visits were

conducted from two perspectives: plot- and individual-level analyses. The plot-level analysis modelled total number of grasshopper visits per plot as a function of the fully crossed factors of the culture type (monoculture with high- or low-nicotine plants, or polyculture with high- and low-nicotine plants) and the number of plants per plot (4, 9 and 16 plants) using GLM. To test the plant frequency-dependent increase in herbivore visits (fitted regression lines in Figure 2), this plot-level analysis was also performed with GLM considering the culture type as a categorical factor and the logarithm number of plants per plot as a continuous variable.

The individual-level analysis used GLMM to test factors affecting the number of grasshopper visits on each individual plant within

the plots. The factors included individual plant variety (high- or low-nicotine variety), culture type (monoculture or polyculture) and number of plants in the plot which were fully crossed and treated as fixed factors. Mixed model analysis was necessary due to repeated measurements of individual plants within a plot. Therefore, we fit a marginal model that accounted for within-plot variation with compound symmetric variance–covariance matrix.

To facilitate interpretation, we present results for a particular factor adjusted for the effects of other components in the statistical models (least-squares means and their standard errors; Milliken & Johnson, 1984). We back-transformed results from the scale of the link function to the original scale of measurement, which results in asymmetrical standard errors.

3 | RESULTS

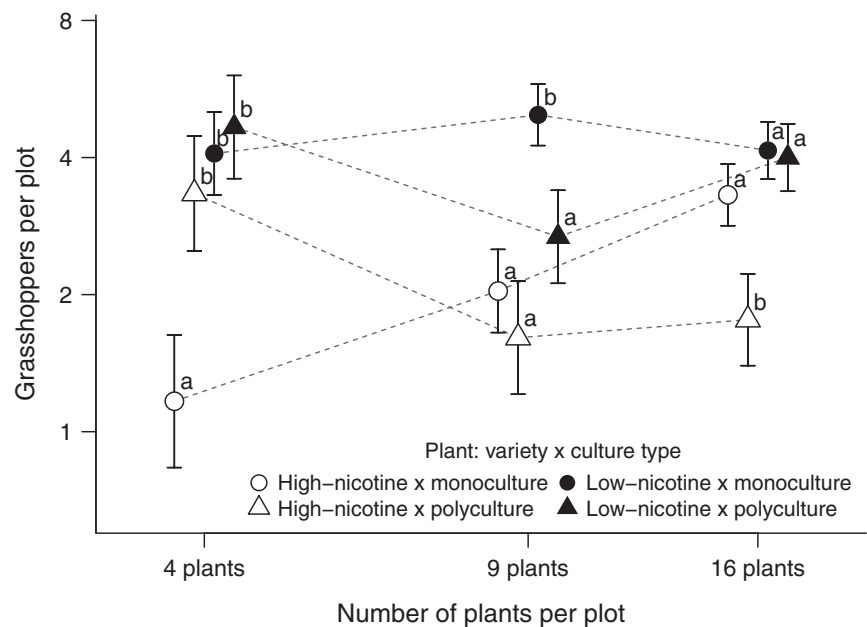### 3.1 | Grasshopper responses to plots (plot-level analysis)

Although the total number of grasshoppers per plot generally increased as the number of plants per plot increased, the responses

TABLE 1 Results of generalized linear models of the effects of culture type (high-nicotine monoculture, low-nicotine monoculture, or polyculture) and number of plants per plot (4 plants, 9 plants or 16 plants) on the total number of grasshoppers per plot of *Nicotiana tabacum* plants (plot-level analysis)

Factor	df	F value
Culture type	2, 18	13.86**
Number of plants per plot	2, 18	61.96**
Culture type × Number of plants per plot	4, 18	3.90*

**$p < .001$, *$p < .05$.

of grasshoppers were strongly affected by an interaction between number of plants and culture type (Figure 2; Table 1). The 16-plant plots had a similar number of grasshoppers among culture types (mean of 55.3, lower SE = 4.19, upper SE = 4.54). In contrast, the number of grasshoppers in the 4-plant plots was 3.5 times greater in the low-nicotine monoculture and polyculture than in the high-nicotine monoculture. In the 9-plant plots, the total number of grasshoppers was 3.4 times greater in low-nicotine monoculture than in high-nicotine monoculture and polyculture. In the analysis treating the number of plants per plot as a continuous variable, effects of both culture types, logarithm number of plants on number of grasshoppers and their interaction were significant (culture type: $F_{2,21}$ = 7.88, $p < .01$, logarithm number of plants: $F_{1,21}$ = 115.17, $p < .01$, interaction term: $F_{2,21}$ = 5.96, $p < .01$). Specifically, the total number of grasshoppers increased proportionally with the logarithm number of plants in low-nicotine monoculture and polyculture plots (Figure 2; partial coefficient, $b + SE$ = 1.00 ± 0.17 for low-nicotine monoculture and 0.80 ± 0.17 for polyculture; comparison with $b = 1$, t_{21} = 0.004, $p > .1$, t_{21} = −1.13, $p > .1$, respectively), whereas it increased proportionally more with increasing logarithm number of plants in high-nicotine monoculture plots (Figure 2; partial coefficient, $b + SE$ = 1.77 ± 0.23; comparison with $b = 1$, t_{21} = 3.38, $p < .001$).

3.2 | Grasshopper responses to individual plants (individual-level analysis)

More detailed analysis of the number of grasshoppers on individual plants detected significant interactive effects of plant variety, culture type and number of plants per plot (Figure 3; Table 2). In general, high-nicotine plants received fewer herbivore visits than low-nicotine ones, but the extent of visit reduction differed

FIGURE 3 Effects of plant variety (high- or low-nicotine variety), culture type (monoculture or polyculture) and number of plants in the plot (i.e. 4, 9 and 16 plants) on least-squares mean (± SE) number of grasshoppers on individual *Nicotiana tabacum* plants (individual-level analysis). Open circles and triangles indicate numbers of grasshoppers on high-nicotine plants in monoculture and polyculture plots respectively. Closed symbols indicate those on low-nicotine plants (circle; monoculture plots, triangle; polyculture plots). Least squares means with different small letters are significantly different within plots with same number of plants (i.e. 4, 9 and 16 plants). Note the logarithmic scaling of the ordinate

TABLE 2 Results of generalized linear mixed models of the effects of plant variety of individual plants (high- or low-nicotine variety), plot culture type (monoculture or polyculture), and number of plants per plot (4 plants, 9 plants or 16 plants) on the total number of grasshoppers on individual plants of *Nicotiana tabacum* (individual-level analysis)

Factor	df	F value
Plant variety (Variety)	1, 83.06	30.56**
Culture type of the plot (Culture type)	1, 25.55	0.15
Number of plants per plot	2, 23.07	0.77
Variety × Culture type	1, 83.06	0.92
Variety × Number of plants	2, 75.64	0.51
Culture type × Number of plants	2, 23.07	4.27*
Variety × Culture type × Number of plants	2, 75.64	3.88*

**p < .001, *p < .05.

depending on the culture type and number of plant per plot. First, effects of plant variety and culture type on herbivore visits differed among plots with different number of plants. In the 4-plant plots, plants in high-nicotine monocultures (i.e. open circles, Figure 3) received 71% fewer grasshoppers than other variety x culture type treatments, indicating that high-nicotine plants decreased grasshoppers to neighbouring plants only in high-nicotine monoculture. In the 9-plant plots, plants in low-nicotine monoculture (i.e. closed circle, Figure 3) received 135% more grasshoppers than other variety × culture type treatments, indicating that the presence of high-nicotine plants reduced the number of grasshoppers on neighbouring plants more than the absence of high-nicotine plants. Surprisingly, in the 16-plant plots, high-nicotine plants in polyculture plots (i.e. open triangle, Figure 3) received 53% fewer grasshopper visits than other plots, including high-nicotine monoculture plots.

Second, the effect of number of plants per plot on herbivore visits differed between plant varieties and culture types. In monoculture plots (i.e. open circles, Figure 3), the number of grasshoppers on high-nicotine plants increased as the number of plants per plot increased (multiple comparisons between the 4-plant and 16-plant plots; $t_{24.68}$ = 2.82, p < .01, the 4-plant and 9-plant plots; $t_{24.05}$ = 1.41, p = .17, and the 9-plant and 16-plant plots; $t_{16.47}$ = 1.86, p = .08). In contrast, grasshopper numbers on high-nicotine plants in polyculture plots (i.e. open triangles, Figure 3) tended to decrease as the number of plants per plot increased (multiple comparisons between the 4-plant and 9-plant plots; $t_{25.67}$ = 1.79, p = .08, the 4-plant and 16-plant plots; $t_{53.72}$ = 1.72, p = .09, and the 9-plant and 16-plant plots; $t_{25.67}$ = 0.25, p = .80). No significant differences in grasshopper visits were found in low-nicotine plants among 4-plant, 9-plant and 16-plant plots (multiple comparisons among plots; t < 0.85, p > .4). Thus, high-nicotine plants affected grasshopper visits differently depending on culture type and number of plants, suggesting that the high-nicotine plant plays a key role in determining the intraspecific neighbourhood effects in a context dependence.

4 | DISCUSSION

4.1 | Density-dependent intraspecific neighbourhood effects

We expected that neighbourhood effects should be altered by plant traits because herbivore responses to host plant cues are highly variable (Hambäck & Englund, 2005). In our study, nicotine as a defensive trait evidently caused intraspecific neighbourhood effects that suppressed herbivore visits, but the neighbourhood effects varied with increasing the patch (plot) size plants and differed among culture types. Our experimental treatment varied simultaneously in number of plants per plot and plant spacing within a plot. Therefore, we discuss potential mechanisms to explain the variations in herbivore plant selection at patch level, which were driven by density of high-nicotine plants and/or patch properties, such as frequency of high- and low-nicotine plants and plant spacing within a patch.

There are two non-exclusive mechanisms that could be responsible for the neighbourhood effect of high-nicotine plants: the repellent hypothesis and the decoy hypothesis (Atsatt & O'Dowd, 1976; Tahvanainen & Root, 1972). The repellent hypothesis predicts that highly defended plants (e.g. less palatable plants) are avoided by herbivores, hence both palatable and non-palatable plants growing together with highly defended plants in a patch are also avoided due to patch avoidance by herbivores. In the four- and nine-plant plots in this study, the repellent effects of nicotine on herbivores were reinforced by the aggregation of high-nicotine plants (i.e. high density and/or plant-spacing; Figure 3), which is consistent with previous studies (Finch & Collier, 2000; Ida et al., 2018). There is another possibility that reduced herbivore visits due to aggregation of high-nicotine plants were driven by closer distance between the plants if herbivore selection is based on relatively small area. However, this possibility is unlikely to occur because there were no significant differences in grasshoppers visits between high-nicotine plants in four- and nine-plant plots, irrespective of different plant spacing (Figure 3). In contrast, in the 16-plant plots, high-nicotine plants in a monoculture did not reduce herbivore visits. This discrepancy may be caused by patch-leaving decision of herbivores. Long-distance movement by herbivores such as grasshoppers just after finding non-palatable plants could cause neighbouring plants to repel herbivores, thereby reducing the likelihood of damage to neighbourhoods in the patch (Hambäck et al., 2014; Ida et al., 2018). However, increasing number of plants within a plot (i.e. patch) also induces aggregated distribution of host plants, suggesting that it enhances 'patch potential' (McNamara, 1982; Verschut, Becher, Anderson, & Hambäck, 2016) through a decrease in movement distance between plants (i.e. plant-spacing effect) and/or an increase in patch-level foraging efficiency owing to abundant foods (i.e. density effect). Therefore, herbivores may stay longer in the patch (i.e. resource concentration; Root, 1973) and delay a patch-leaving decision (Stephens & Krebs, 1986; Waage, 1979). Thus, neighbourhood effects caused by the repellent effects of nicotine varied depending on aggregation of highly

defended plants per patch. Future studies that disentangle density effects from plant-spacing effects would provide valuable insights to assess fully fine-scale neighbourhood effects.

Alternatively, the decoy hypothesis predicts that plants are avoided by herbivores if they neighbour more palatable plants. This situation would have occurred only in high-nicotine plants in polyculture in the 16-plant plot (Figure 3). The decoy effect predicts that herbivores move within a patch, and hence less palatable plants (i.e. high-nicotine plants) receive less herbivory because low-nicotine plants work as 'decoy'. However, our study did not detect increased herbivore visits on low-nicotine plants in polyculture plots as the decoy hypothesis predicts. Instead, heterogeneity in nicotine level may stimulate movement of herbivores (Karban, 2011) and suppress herbivore visits to high-nicotine plants compared to low-nicotine plants. For instance, a recent study using *Drosophila melanogaster* found that flies visited more favourable resources within a patch with heterogeneous resources (Verschut et al., 2016). Therefore, this decoy effect may be weak for herbivore suppression. Based on the implication above, our results from polyculture plots demonstrated that both hypotheses were conditionally acceptable.

Taken together, our study clearly illustrated that the neighbourhood effect could arise from simultaneous operation of repellent, resource concentration and decoy hypotheses. As a result, intraspecific neighbourhood effects varied unimodally with respect to aggregation of high-nicotine plants, with a peak at intermediate density. Although a number of studies have discussed potential mechanisms of neighbourhood effects (mostly interspecific effects; Andow, 1991; Barbosa et al., 2009), a very few studies have reported the operation of multiple mechanisms that simultaneously work in a spatial context (but see Coverdale et al., 2018; Kim & Underwood, 2015). Kim and Underwood (2015) stressed that frequency-dependent neighbourhood effects could be nonlinear. Likewise, our study detected that density-dependent neighbourhood also could be nonlinear because the manner of density dependence could differ among different mechanisms, which had such different outcomes in different densities. The strength of combined effects of multiple mechanisms of neighbourhood effect caused by spatial structure at patch level would determine positive or negative net intraspecific neighbourhood effects, which would impose heterogenetic neighbourhood effects within a population. In fact, meta-analysis by Champagne, Tremblay, and Cote (2016) showed that strength of neighbourhood effects increased with decreasing plot size. Such neighbourhood effects at patch level would strongly influence the spatial distribution of herbivores within a plant population and presumably subsequent plant performances.

4.2 | Population-level consequences of intraspecific neighbourhood effects

Although recent studies have argued the importance of neighbourhood effects on plant performance (e.g. herbivore escape

and subsequent growth) within a population (e.g. Hahn & Orrock, 2016; Kim & Underwood, 2015), underlying mechanisms are still poorly understood (Underwood et al., 2014). This is because most studies have only focused on associational effects of interspecific interactions independent of population-level consequences of a single plant species, ignoring how neighbourhood effects vary with the number of conspecific plants (but see Bustos-Segura, Poelman, Reichelt, Gershenzon, & Gols, 2017). Our study that focused on the intraspecific interactions is the first step towards understanding how neighbourhood effects involving two genotypes with different level of the defensive chemical might scale up to plant population-level consequences.

In nature, individual plants are not always distributed uniformly within a population (Sagarin & Gaines, 2002) but grow with mixture of different genotypes (Agrawal et al., 2002). Hence, neighbourhood effects would vary due to patch-level properties. Thus, we need to incorporate plant genetic variation and spatial structure into the intraspecific neighbourhood effect. This suggests that population-level consequences reflect not only the sum of interactions via defensive traits between individual plants and herbivores, but also the sum of their interactions at a patch level. Therefore, the consequences largely rely on underlying mechanisms of neighbourhood effects.

There are two contrasting mechanisms generating different consequences of neighbourhood effects for the plant population. One mechanism involves effects of increasing number of plants per patch, such as attractant effects (resource concentration hypothesis: Kery, Matthies, & Fischer, 2001; Ostergard & Ehrlén, 2005; Root, 1973; Sholes, 2008) and resource-dilution effects (resource dilution hypothesis; Otway et al., 2005). For instance, the resource dilution hypothesis predicts that a greater number of plants within a patch would dilute more per capita herbivory (Hambäck et al., 2014; Otway et al., 2005). Hence, the mean-value of herbivory and subsequent negative effects on survival and growth of the damaged plants in the patch would decrease with increasing plant density, although stochastic choice by herbivores determines whether an individual plant can escape from herbivory. Such neighbourhood effects create lottery competition for escape from herbivores among plant neighbours. This may lead to homogeneity in plant traits within each patch, such that plants can equally contribute to population persistence. Another mechanism involves frequency-dependent effects (via variations in cue traits (e.g. defensive traits) for herbivores), which have been emphasized by the repellent and decoy hypotheses (Atsatt & O'Dowd, 1976; Tahvanainen & Root, 1972). Whether such neighbourhood effects incur benefits or costs for plants with high- and low-palatability (or low- or high-defended plants) depends on herbivore behaviour (e.g. within-patch or between-patch movement). Plant defence against herbivores imposes a high cost for chemical production, resulting in decreased growth and reproduction (Agrawal, 2000; Agrawal et al., 2002). If low-defended plants can successfully escape from herbivory under the patronage of neighbouring high-defended plants, they can effectively grow

and reproduce without any costs against herbivores. Therefore, population persistence would be promoted by variations in plants with different defensive traits, reflecting the trade-off between defence against herbivore and growth and/or reproduction.

5 | CONCLUSIONS

Likelihood of herbivore visits clearly declines when an individual plant has defensive traits, such as chemical compounds (e.g. *Nicotiana* species: Kessler, Halitschke, & Baldwin, 2004). Furthermore, our study revealed that chemical defensive traits do not always reinforce suppression of herbivore visits because neighbourhood effects are variable due to properties of plant patches, such as the aggregation of highly defensive plants (i.e. high density and/or plant spacing) and the frequency (i.e. relative density) of neighbours with different defensive traits. A patchy or clumped distribution of plants commonly occurs in nature, resulting in variable local plant–plant interactions. Our findings indicate that exploring intraspecific genetic and/or phenotypic diversity and spatial structures of individual plants is critical for better understanding of the herbivore's plant selection and plant performances as population-level consequences of spatially structured plant–herbivore interactions.

AUTHORS' CONTRIBUTIONS
M.T., T.O. and T.Y.I. contributed equally to the development of ideas and research plans and writing of the paper, while M.T. and T.Y.I. led the data collection and analysis efforts.

DATA AVAILABILITY STATEMENT
Data deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.zkh18936b (Ida, Tamura, & Ohgushi, 2019).

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