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Strong but opposing effects of associational resistance and susceptibility on defense phenotype in an African savanna plant

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The susceptibility of plants to herbivores can be strongly influenced by the identity, morphology and palatability of neighboring plants. While the defensive traits of neighbors often determine the mechanism and strength of associational resistance and susceptibility, the effect of neighbors on plant defense phenotype remains poorly understood. We used field surveys and a prickle-removal experiment in a semi-arid Kenyan savanna to evaluate the efficacy of physical defenses against large mammalian herbivores in a common understory plant, *Solanum campylacanthum*. We then quantified the respective effects of spinescent *Acacia* trees and short-statured grasses on browsing damage and prickle density in *S. campylacanthum*. We paired measurements of prickle density beneath and outside tree canopies with long-term herbivore-exclusion experiments to evaluate whether associational resistance reduced defense investment by decreasing browsing damage. Likewise, we compared defense phenotype within and outside pre-existing and experimentally created clearings to determine whether grass neighbors increased defense investment via associational susceptibility. Removing prickles increased the frequency of browsing by ~25%, and surveys of herbivory damage on defended leaves suggested that herbivores tended to avoid prickles. As predicted, associational resistance and susceptibility had opposing effects on plant phenotype: individuals growing beneath *Acacia* canopies (or, analogously, within large-herbivore exclosures) had a significantly lower proportion of their leaves browsed and produced ~ 70–80% fewer prickles than those outside refuges, whereas plants in grass-dominated clearings were more heavily browsed and produced nearly twice as many prickles as plants outside clearings. Our results demonstrate that associational resistance and susceptibility have strong, but opposing, effects on plant defense phenotype, and that variable herbivore damage is a major source of intraspecific variation in defense phenotype in this system.

Keywords: associational effects, associational refuge, herbivory, physical plant defenses, *Solanum incanum*, spines and thorns

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Introduction

Intrinsic plant defenses – including physical, chemical and biological defenses – reduce tissue loss and mitigate the deleterious effects of herbivory on plant fitness (Herms and Mattson 1992). Investment in intrinsic defenses is highly variable within and among species, and understanding the causes and consequences of this variation has been a central goal in the study of plant–herbivore interactions for decades (Coley et al. 1985, Burkepile and Parker 2017). In addition to modulating herbivory damage to the plants that produce them, defenses and other plant traits can also increase (associational susceptibility) or decrease (associational resistance) herbivory on neighboring plants (McNaughton 1978, Hay 1986, Barbosa et al. 2009, Underwood et al. 2014). For example, Baraza et al. (2006) reported that large-mammal browsing on palatable maple *Acero palus* subsp. *granatense* saplings decreased as neighbors became more unpalatable and better defended. Efforts to link the phenotypes of neighbors with the mechanisms and outcomes of associational resistance and susceptibility have been a mainstay of studies of associational effects (Baraza et al. 2006, Kim and Underwood 2015).

Although it is widely accepted that plants modulate their defense phenotypes to match their risk of being browsed (Karban and Baldwin 1997), and that neighbors can substantially alter browsing risk (Barbosa et al. 2009), few studies have investigated the interaction between associational effects and induced resistance (Coverdale et al. 2018). Of those that have explored this interaction, the majority have investigated how defense induction affects the magnitude or direction of the associational effects generated by the induced plant (e.g. soybeans: Underwood et al. 2005; post-agricultural fields: Kim 2017; boreal forests: Benevenuto et al. 2018). However, neighbors may also indirectly affect the defense phenotype of nearby plants by increasing or decreasing the cues (e.g. physical damage, chemical cues, volatile emissions) necessary for defense induction (Arimura et al. 2000, Coverdale et al. 2018). Given the ubiquity of associational effects and induced resistance in plant communities, association-driven shifts in defense investment may be a widespread source of intraspecific variation in defense phenotype across ecosystems (Hahn and Maron 2016) and may exert significant effects on herbivore preference and performance, as well as on plant survival and fitness (Underwood et al. 2014, Burkepile and Parker 2017).

We investigated whether associational resistance and associational susceptibility cause predictable shifts in plant defense phenotype in a semi-arid Kenyan savanna. At our study site, proximity to physically defended *Acacia* trees significantly reduces herbivory on understory plants (Coverdale et al. 2016, 2018), whereas plants growing in clearings dominated by palatable, short-statured grasses are exposed to more intense mammalian herbivory (Augustine and McNaughton 2006, Veblen 2012, Ford et al. 2014). Here, we quantified the effects of associational resistance (occurring at the scale of individual *Acacia* tree canopies; $\sim$ 5–20 m²) and susceptibility (occurring at the scale of treeless, grassy clearings; $\sim$ 5000–20 000 m²) on the defense phenotype of *Solanum campylacanthum*. This abundant, perennial subshrub produces recurved prickles ($\sim$ 5 mm long) along the leaf midrib and stems as a primary physical defense (Pringle et al. 2014). Because the efficacy of physical defenses in understory plants has not been as well characterized as those of trees and shrubs in African savannas (Young 1987, Gowda et al. 2003, Charles-Dominique et al. 2016), we began by investigating the hypothesis (H_1) that *S. campylacanthum* prickles deter large mammalian browsers. We then used a combination of surveys and experimental manipulations to characterize patterns of defense investment across associational contexts. Specifically, we hypothesized that (H_2) *S. campylacanthum* would incur reduced browsing damage beneath *Acacia* canopies owing to physical inhibition of herbivores (i.e. associational resistance conferred by neighboring trees), and that plants within associational refuges would be less defended than conspecifics growing outside tree canopies owing to increased browsing damage (and defense induction) in the latter. Likewise, we hypothesized (H_3) that *S. campylacanthum* would incur greater browsing damage within treeless clearings (i.e. associational susceptibility conferred by neighboring grasses), and that plants in these areas would be more defended than conspecifics growing outside clearings owing to increased browsing damage and defense induction. For each hypothesis, we used long-term, large-scale experimental manipulations to isolate the effects of browsing pressure on defense phenotype from those of abiotic conditions.

Material and methods

Study site and statistical analyses

The Mpala Research Centre and Conservancy (MRC) encompasses $\sim$20 000 ha of thorn-scrub savanna and dry woodland in Kenya's Laikipia County (0°36′4″N, 36°87′8″E), two-thirds of which is underlain by red sandy loams (Pringle et al. 2016). The plant community on this soil type consists of a discontinuous overstory dominated by spinescent *Acacia* trees (*A. brevispica*, *A. etbaica* and *A. mellifera*) and an understory comprising various species of grasses, forbs and subshrubs (Goheen et al. 2013). Of the latter, many species, including *Solanum campylacanthum* Hochst. ex A. Rich (frequently misidentified as *S. incanum* L.: Knapp et al. 2013) are physically and/or chemically defended against large mammalian browsers: *S. campylacanthum* produces recurved prickles along the stem, petiole and midrib characteristic of 'spiny' *Solanum* species in the subgenus *Leptostemonum* (Levin et al. 2006; Fig. 1A, Supplementary material Appendix 3 Fig. A1), along with steroidal glycoalkaloids that are toxic to humans and livestock (Thaiyah et al. 2011), but do not strongly deter wild browsers (Pringle et al. 2014). MRC supports more than twenty species of wild large mammalian herbivores, including elephant *Loxodonta africana*, impala *Aepyceros melampus*
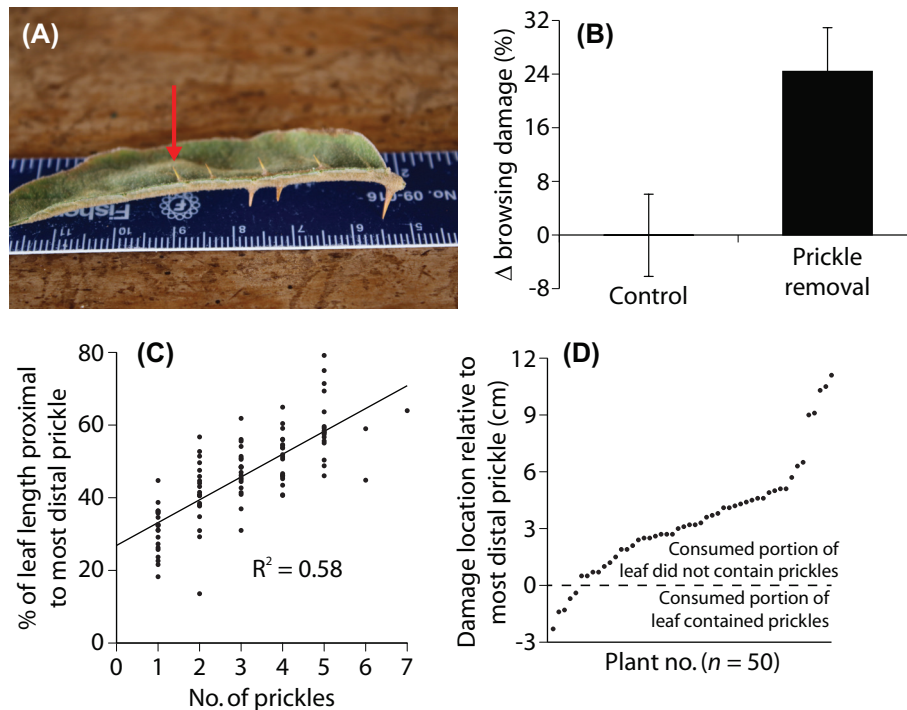
Figure 1. Evidence for the efficacy of *S. campylacanthum* prickles against large mammalian browsers. (A) A bisected *S. campylacanthum* leaf showing prickles along the top and bottom of the leaf midrib. Arrow indicates most distal prickle. (B) Changes in browsing damage on *S. campylacanthum* one month after experimental removal (black bar) of prickles. (C) Relationship between number of prickles/leaf and proportion of the leaf proximal to the most distal prickle, suggesting that plants with more prickles have less undefended tissue at the leaf tip. (D) Distribution of browsing damage relative to the most distal prickle on 50 *S. campylacanthum* leaves. Values above the dashed line indicate that browsers consumed only leaf parts lacking prickles. Scale bar (cm) for reference; data are means ± 1 SEM.

and dik-dik *Madoqua cavendishi*, as well as herds of domesticated cattle *Bos indicus*, sheep *Ovis aries*, goat *Capra hircus* and camel *Camelus dromedarius*. *Solanum campylacanthum* is eaten by browsing and mixed-feeding ungulates across the body-size spectrum, including elephant, impala and dik-dik (Pringle et al. 2014, Kartzinel et al. 2015). Livestock at MRC are tended using modified pastoralist methods, including the corralling of individuals overnight in temporary enclosures ('bomas') constructed from spiny *Acacia* branches or metal fencing. Abandoned boma sites develop into nutrient-rich, grass-dominated 'glades' (Porensky and Veblen 2015), which have lower tree cover and higher soil nutrients than the surrounding savanna habitat and are hotspots of herbivore activity, owing to both the greater nutritional quality of forage and the lower risk of predation conferred by higher visibility (Young et al. 1995, Augustine 2004, Ford et al. 2014, Riginos 2015). Glades should therefore be areas where subshrubs like *S. campylacanthum* experience associational susceptibility, as a result of their increased apparency amidst grass neighbors and the greater herbivore activity that these clearings attract.

To investigate the effects of associational resistance on intraspecific variation in defense investment in *S. campylacanthum*, we used two long-term herbivore exclosure experiments (the Glade Legacies and Defaunation Experiment, hereafter 'GLADE': Augustine and McNaughton 2006, and the Ungulate Herbivory Under Rainfall Uncertainty experiment,

hereafter 'UHURU': Goheen et al. 2013). Briefly, the UHURU and GLADE experiments use similar fencing treatments to exclude large mammalian herbivores (Goheen et al. 2018). The GLADE experiment, initiated in 1999 (Augustine and McNaughton 2006), consists of paired unfenced control and fenced exclosure plots (excluding all herbivores >5 kg); we collected data from two paired plots in southern MRC. The UHURU experiment, initiated in 2008 (Goheen et al. 2013), includes analogous unfenced and fully fenced exclosure treatments; we collected data from paired sites (n = 3 pairs/region) in northern and southern MRC (see Supplementary material Appendix 1 for details on site locations). We used both exclosure experiments to test the hypothesis (H_2) that the anti-herbivore aspect of association with *Acacia*, and not the effect of trees on abiotic conditions in the understory, decreases defense investment in associated *S. campylacanthum*.

To investigate the effects of associational susceptibility on intraspecific variation in defense investment in *S. campylacanthum*, we used a large-scale artificial clearing experiment (Ford et al. 2014). Between October 2011 and February 2012, five 0.5-ha plots (hereafter 'experimental clearings') were cleared of all trees in central and northern MRC; each experimental clearing was paired with an adjacent, unmanipulated patch of equivalent size (Ford et al. 2014). Cleared plots are comparable to glades in size and tree cover, and both are dominated by herbaceous understory plants. However, in

contrast to glades, where grasses have ~66% greater [N] and 160% greater [P] owing to the legacy of concentrated dung and urine deposition by corralled cattle (Augustine et al. 2011), cleared plots are not nutrient enriched. Although it is possible that sustained elevated utilization by wild ungulates such as impala – which have been shown to aggregate in cleared plots to mitigate predation risk (Ford et al. 2014) – would eventually increase nutrient concentrations, marked accumulation is unlikely to have occurred by the time of our study (which was conducted ~3.5 years after the initial clearing). Therefore, clearing should isolate the effects of treelessness from those of the nutrient enrichment found in glades. For the purposes of this study, we used the experimental clearings to test the hypothesis that association with short-statured grass neighbors increases defense investment in glades, irrespective of the changes in nutrient availability and other abiotic conditions (e.g. soil compaction) that accompany glade formation.

The identity and abundance of dominant browsers, as well as soil-texture and nutrient conditions, are broadly comparable across all three long-term experiments, which span ~25 km along a north–south axis at MRC (Supplementary material Appendix 1). Because the northern region of MRC has historically received ~30% less rainfall per year than the southern region (Goheen et al. 2013, Louthan et al. 2013, Kartzinel et al. 2014), we treated region as a fixed effect in all analyses of data that span the full rainfall gradient. However, because region (representing rainfall) ultimately did not have a significant effect on browsing damage or defense phenotype in any of our analyses (a result consistent with multiple previous studies at MRC: Goheen et al. 2013, Ford et al. 2014, Pringle et al. 2014, but see Louthan et al. 2013, 2014, 2017) we do not present or discuss those results in the main text; full model outputs for all analyses are instead presented in Supplementary material Appendix 2. For surveys and experiments within the GLADE and UHURU plots, pre-existing glades and artificial clearings, we surveyed 10–15 individual plants per plot; individual estimates of browsing damage or defense investment were averaged within each plot or clearing (following Pringle et al. 2014, Long et al. 2017) and plot-level averages were compared with ANOVA ($\alpha = 0.05$) in R (ver. 3.3.2; <www.r-project.org>). For analyses of all survey and experimental data outside experimental exclosures and clearings, individual plants were treated as independent replicates. A full description of the predictions, design and analysis of all experiments and surveys is presented in Table 1, and the location of all experiments and surveys in this study can be found in Supplementary material Appendix 1. A synopsis of long-term experimental infrastructure at MRC is provided by Goheen et al. (2018). All data presented are means ± SEM.

H$_1$. Prickles reduce browsing and constrain herbivore damage

Although spines and thorns are known to deter large mammals from browsing savanna trees and shrubs (Cooper and Owen-Smith 1986, Charles-Dominique et al. 2016), the efficacy of physical defenses against large browsers in understory plants has received less study. We therefore tested 1) whether prickles are an effective deterrent against herbivores, 2) whether leaves with more prickles have less undefended area at the leaf tip and 3) whether the presence of prickles confined herbivory to the tips of leaves. To determine if prickles are an effective anti-herbivore defense, we selected 80 *S. campylacanthum* in southern MRC and randomly assigned half to a prickle-removal treatment: all prickles were removed from stems, petioles and leaf midribs using scissors, with care taken to minimize damage to other tissues (Supplementary material Appendix 3 Fig. A2). Control plants (n = 40 plants) were not manipulated. Changes in browsing damage over one month were compared across treatments with one-factor ANOVA. Damage caused by large mammalian herbivores was readily distinguishable from the small incisions required to remove prickles.

Because *S. campylacanthum* prickles tend to be located along the petiole and proximal midrib of leaves (Fig. 1A, Supplementary material Appendix 3 Fig. A1), the distal portion of leaves typically lacks physical defenses. To determine if leaves with more spines had a smaller proportion of their total length beyond the most distal prickle, we surveyed the number and distribution of prickles on leaves from 100 *S. campylacanthum*. For each plant, we haphazardly selected a single leaf, counted the total number of prickles on the leaf midrib and petiole, and measured the total leaf length and the length from the leaf base to the most distal prickle. We then calculated the proportion of leaf length beyond the most distal prickle and compared this to the number of prickles with linear regression.

Next, we surveyed the distribution of prickles along pairs of browsed and unbrowsed leaves to test whether herbivory tended to occur in the undefended distal portion of leaves. For each of 50 *S. campylacanthum* in southern MRC, we identified a browsed leaf and the nearest, adjacent, unbrowsed leaf of similar size, using maximum leaf width as a proxy for leaf area (similarly sized leaves from the same plant tend to have similar numbers and distributions of prickles). For browsed leaves we measured the total length (i.e. leaf base to browsing scar), and for unbrowsed leaves we measured the distance between the leaf base and the most distal prickle. We then calculated the difference between browsed and unbrowsed leaf lengths for each pair; positive values of this metric indicate that browsing occurred beyond the presumed location of the most distal prickle (i.e. that the consumed portion of the leaf did not contain prickles), whereas negative values suggest that herbivores removed tissue containing at least one prickle. Data were analyzed with a one-tailed t-test.

Positive values of the above metric could result from 1) herbivore preference for leaf tips regardless of the location of prickles, 2) bite-size limitation for small herbivores such as dik-dik or 3) avoidance of prickles by herbivores. To differentiate between these mechanisms, we drew upon the observation that leaves with fewer prickles tend to have a greater distance between the leaf tip and the most distal prickle (Fig. 1C). If herbivores are constrained by bite size or

Table 1. Description of experiments and surveys addressing the efficacy of prickles against herbivores (H_1) and the effects of associational resistance (H_2) and susceptibility (H_3) on browsing damage and defense phenotype. [1]Unbrowsed leaves. [2]Browsed leaves. [3]Full exclosure + unfenced control plots. [4]Paired glade and non-glade sites. [5]Paired experimental clearing and control sites. [†]One-factor. [‡]Two-factor. [Δ]Individual plants treated as independent replicates. [Σ]Individual plants averaged within experimental exclosure, clearing or glade.

Hypothesis	Component	Prediction	Traits measured	Location	Replication	Analysis	Figure
H_1: Prickles reduce browsing and constrain herbivore damage on S. campylacanthum	Prickle removal experiment	Prickle removal increases browsing	Change in browsing damage	southern MRC	n = 40 plants/treatment	ANOVA[†Δ]	Fig. 1B
	Prickle distribution survey	Leaves with more prickles have less undefended tissue at the leaf tip	No. prickles/leaf[1] Total leaf length[1] Length from base to most distal prickle[1]	southern MRC	n = 100 plants×one leaf/plant	linear regression[Δ]	Fig. 1C
	Herbivory location survey	Herbivory is restricted to the undefended portion of leaf tips	Total leaf length[1] Length from base to browsing scar[2] Difference in leaf lengths	southern MRC	n = 50 plants×two leaves/plant	one-tailed t test[Δ]	Fig. 1D
H_2: Associational resistance reduces defense investment in S. campylacanthum	Browsing damage survey	Associational resistance reduces browsing	Browsing damage	southern MRC	n = 60 plants/associational status×five leaves/plant	ANOVA[†Δ]	Fig. 2A
	Defense investment survey	Associational resistance reduces defense investment	Prickle density	northern and southern MRC	n = 60 plants/associational status×five leaves/plant	ANOVA[‡Δ]	Fig. 2B
	Defense investment survey (exclosures)	Reduced resistance reduces defense investment	Prickle density	southern GLADE and UHURU plots[3]	GLADE n = 15 plants/plot UHURU n = 10 plants/plot	ANOVA[‡Σ]	Fig. 2C
H_3: Associational susceptibility increases defense investment in S. campylacanthum	Browsing damage survey	Associational susceptibility increases browsing	Browsing damage	natural glades in northern and southern MRC	n = 10 plants/habitat×six sites	ANOVA[‡Σ]	Fig. 3A
	Defense investment survey	Associational susceptibility increases defense investment	Prickle density	natural glades in northern and southern MRC	n = 10 plants/habitat×six sites[4]	ANOVA[‡Σ]	Fig. 3B
	Defense investment survey (clearings)	Associational susceptibility increases defense investment	Prickle density	artificial clearings	n = 10 plants/clearing×five sites[5]	ANOVA[†Σ]	Fig. 3C

prefer leaf tips, we expect a similar amount of leaf tissue to be removed from the leaf tip regardless of the location of the most distal prickle; leaves with fewer prickles would therefore have a greater distance between the browsing scar and the most distal prickle. Alternatively, if browsers avoid prickles, we expect herbivores to browse up to the most distal prickle, regardless of where that prickle occurs on the leaf; the distance between the browsing scar and the most distal prickle would therefore be similar across leaves with different numbers of prickles. Data were analyzed with linear regression (n = 50 leaves).

H₂. Associational resistance reduces defense investment

To determine whether spinescent *Acacia* trees provide associational refuges for *S. campylacanthum*, we measured browsing damage on 120 associated and unassociated individuals (n = 60 plants/associational status) in southern MRC; associated individuals were directly beneath *Acacia* canopies, the branches of which typically extended to within 50 cm of the ground, whereas unassociated plants were always >1 m from the nearest tree canopy. For each plant, we haphazardly selected five leaves, scored them as browsed or unbrowsed, and calculated an average browsing score (0–100%) for each plant. Signs of large mammalian browsing were clearly distinguishable from insect damage. Differences in browsing damage were analyzed with one-factor ANOVA.

We then quantified the defense phenotype of another 120 associated and unassociated plants by counting the number of prickles on five haphazardly selected leaves per plant. Plants were evenly divided between areas in northern and southern MRC (n = 30 plants/associational status/region), and defense investment was approximated as the average number of prickles per leaf for each plant. Differences in defense investment between associated and unassociated plants were compared with a two-factor ANOVA, with habitat (associated versus unassociated), region (north versus south), and their interaction as fixed effects.

Refuge and non-refuge habitats differ not only in browsing pressure, but also various abiotic conditions (e.g. photosynthetically available radiation, soil moisture, soil nutrients) which may contribute to variation in defense phenotype (Coverdale et al. 2016, 2018). Thus, to assess whether variation in herbivory damage alone was sufficient to drive intraspecific patterns of defense investment within versus outside associational refuges, we measured average prickle density on unassociated *S. campylacanthum* plants in the fenced exclosure plots and unfenced control plots of the GLADE (n = 15 plants/plot × two blocks) and southern UHURU (n = 10 plants/plot × three blocks) exclosure experiments. Differences in prickle density were averaged for each plot and analyzed with separate one-factor ANOVA for each exclosure experiment, with exclosure treatment as a fixed effect.

H₃. Associational susceptibility increases defense investment

To determine whether browsing damage was greater within glades (abandoned cattle bomas), we measured browsing damage on 120 *S. campylacanthum* at six glade sites. At each site, plants were measured within a single glade and an adjacent non-glade area of comparable size (n = 10 plants/habitat/site × six sites). Three sites each were located in the northern and southern regions of MRC (Supplementary material Appendix 1). We then quantified prickle density on the same plants to determine whether defense investment was greater within glades. Browsing damage and prickle density data were analyzed using separate two-factor ANOVA, with habitat (glade versus non-glade), region (north versus south) and their interaction as fixed effects; browsing damage and prickle density estimates were averaged for each glade and non-glade area.

Glade and non-glade habitats differ not just in tree density and herbivory pressure (Ford et al. 2014), but also in plant community composition, soil macronutrients, livestock dung deposition rates and arthropod abundance (Augustine 2003, 2004, Veblen 2012, Donihue et al. 2013, Porensky and Veblen 2015), any or all of which might contribute to intraspecific differences in defense investment across habitats. Thus, to isolate the effects of herbivory on plant defense investment in treeless, grass-dominated areas, we surveyed prickle density on 120 *S. campylacanthum* in five experimentally cleared plots and five adjacent unmanipulated control plots (n = 12 plants/habitat/site × five sites); tree removal occurred ~3.5 years prior to our survey (Ford et al. 2014). As noted above, the experimentally cleared plots are superficially similar to glades in that they are dominated by short-statured grasses and are hotspots of herbivore activity (Ford et al. 2014, Riginos 2015), but unlike glades they are not nutrient enriched and do not support greater arthropod densities (Donihue et al. 2013), enabling us to isolate the effects of mammalian herbivory pressure and association with short-statured grasses from those of resource availability. Differences in defense investment were averaged for each experimental clearing and analyzed with one-factor ANOVA, with habitat (experimental clearing versus unmanipulated control plot) as the fixed factor.

Data deposition

Results

H₁. Prickles reduce browsing and constrain herbivore damage

Removing prickles from *S. campylacanthum* increased the number of leaves with browsing damage by 24.4 ± 6.5% (SEM) over one month ($F_{1,78}$ = 7.46, p = 0.008, Fig. 1B),

whereas unmanipulated plants exhibited no net change ($-0.05 \pm 6.1\%$) in browsing damage over the same time period. Leaves with more prickles tended to have a smaller proportion of their length beyond the most distal prickle ($F_{1,98} = 134.7$, $p < 0.0001$, $R^2 = 0.57$; Fig. 1C). On average, browsing damage occurred 3.5 ± 0.42 cm beyond the inferred location of the most distal prickle on browsed leaves ($t_{(49)} = 8.33$, $p < 0.0001$; Fig. 1D) regardless of the location of the most distal prickle (slope: -0.075, $F_{1,48} = 0.078$, $p = 0.78$), suggesting that herbivores browsed up to the most distal prickle rather than removing only the leaf tips. Collectively, these results suggest that prickles are an effective deterrent of large mammalian herbivores, that plants with a greater number of prickles per leaf have less undefended tissue than those with fewer prickles, and that browsing is largely restricted to the leaf tip due to the presence of prickles in more proximal leaf tissue.

H$_2$. Associational resistance reduced defense investment

Association with spinescent *Acacia* trees reduced the average number of *S. campylacanthum* leaves with browsing damage by 80.4% ($F_{1,118} = 19.77$, $p < 0.0001$, Fig. 2A). Moreover, associated plants produced 79.6% fewer prickles on average than did unassociated conspecifics (0.61 ± 0.11 versus 2.97 ± 0.26 prickles per leaf; association effect: $F_{1,116} = 74.79$, $p < 0.0001$, Fig. 2B).

The effects of long-term herbivore exclusion on defense investment were essentially equivalent to those of association with *Acacia* and were consistent across the two independent exclosure experiments (compare Fig. 2B–C). Unassociated *S. campylacanthum* within the then eight-year-old UHURU herbivore exclosure plots produced, on average, 0.78 ± 0.93 prickles per leaf, whereas those in adjacent unfenced control plots produced 2.62 ± 0.18 prickles per leaf ($F_{1,4} = 80.97$, $p = 0.0008$; Fig. 2C). Similarly, unassociated plants within the then 17-year-old GLADE exclosure plots produced, on average 0.51 ± 0.02 prickles per leaf, whereas those in unfenced control plots produced 3.03 ± 0.44 prickles per leaf ($F_{1,2} = 32.56$, $p = 0.029$; Fig. 2C).

H$_3$. Associational susceptibility increased defense investment

Browsing damage on *S. campylacanthum* was approximately five-fold greater within glades than in immediately adjacent non-glade habitat (habitat effect: $F_{1,8} = 225.0$, $p < 0.0001$; Fig. 3A). Variation in defense investment mirrored that of browsing damage between glade and non-glade habitats: *S. campylacanthum* within glades (2.86 ± 0.36 prickles per leaf) had nearly twice as many prickles per leaf as those outside glades (1.54 ± 0.18 prickles per leaf; habitat effect: $F_{1,8} = 12.47$, $p = 0.008$, Fig. 3B).

Patterns of defense investment in experimentally cleared plots were nearly identical to those observed in glades: *S. campylacanthum* within clearings (3.29 ± 0.48 prickles per leaf) invested approximately twice as much in physical defenses as did those outside clearings (1.60 ± 0.21 prickles per leaf; $F_{1,8} = 10.37$, $p = 012$; Fig. 3C).

Discussion

We found that the intensity of browsing by large mammalian herbivores on *S. campylacanthum* was modulated by both intrinsic (i.e. prickles), and extrinsic (i.e. associational effects) defense strategies. Despite the small size of *S. campylacanthum* prickles relative to the native mammalian herbivores (e.g. dik-dik, impala, elephant) known to consume this understory species (Pringle et al. 2014, Kartzinel et al. 2015; Fig. 1A, Supplementary material Appendix 3 Fig. A5), our results suggest that prickles are an effective antiherbivore deterrent: removing prickles increased the number of leaves with browsing damage by ~25% (Fig. 1B), and the majority of browsing occurred on the relatively undefended tips of leaves (Fig. 1D). Although these results are consistent with herbivore avoidance of prickles, we are not able to conclusively rule out the (not mutually exclusive) alternative explanations that bite-size restrictions in the smaller herbivore species (e.g. dik-dik) and/or herbivore preference for leaf tips regardless of prickle location influenced these patterns. However, the consumption of leaf tissue that formerly contained prickles after experimental prickle removal (Supplementary material
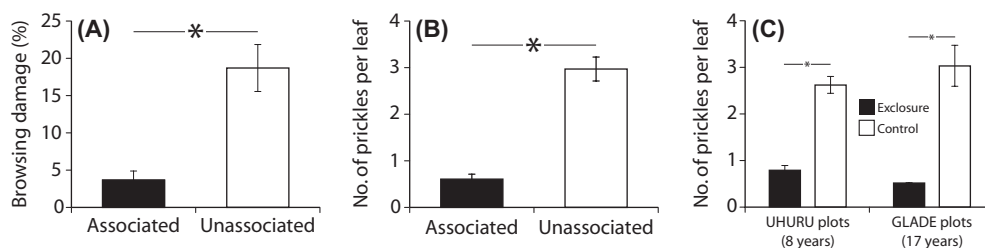

Figure 2. Effects of associational resistance on browsing damage and defense investment. (A) Browsing damage on *S. campylacanthum* within (black bar) and outside (white bar) the refuge habitat beneath spinescent *Acacia* canopies. (B) Defense investment by *S. campylacanthum* within (black bar) and outside (white bar) the refuge habitat beneath *Acacia* canopies. (C) Defense investment by unassociated *S. campylacanthum* within (black bar) and outside (white bar) two long-term herbivore exclosure experiments. Asterisk denotes statistically significant differences ($p \leq 0.05$) between habitats or experimental exclosure treatments; data are means $\pm$ 1 SEM.
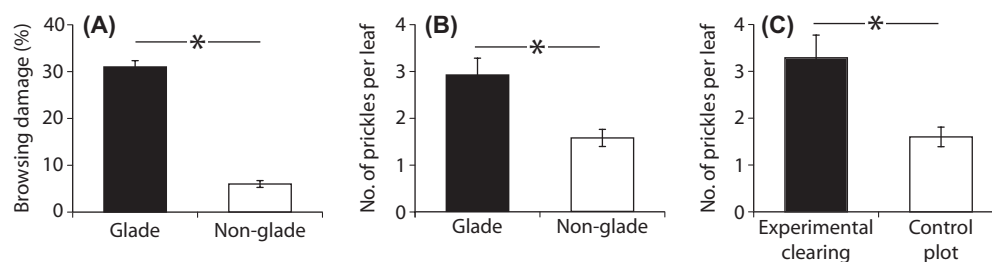
Figure 3. Effects of associational susceptibility (proximity to short-statured grasses) on browsing damage and defense investment in *S. campylacanthum*. (A) Browsing damage on *S. campylacanthum* within (black bar) and adjacent to (white bar) naturally occurring treeless glades. (B) Defense investment by *S. campylacanthum* within (black bar) and adjacent to (white bar) naturally occurring treeless glades. (C) Defense investment by *S. campylacanthum* within (black bar) and adjacent to (white bar) experimentally cleared plots. Asterisk denotes statistically significant differences (p ≤ 0.05) between habitats or experimental clearing treatments; data are means ± 1 SEM.

Appendix 3 Fig. A3) and the consistent location of browsing scars ~3.5 cm from the most distal prickle (regardless of the proximity of that prickle to the leaf tip) suggest that herbivore avoidance of prickles is the most probable explanation for observed patterns of leaf damage.

As predicted, we also found that browsing intensity was decreased by proximity to spinescent *Acacia* trees: relative to conspecifics growing adjacent to tree canopies, individuals growing beneath tree canopies (and, analogously, within long-term herbivore exclosures) experienced ~80–100% less browsing damage. Because the understory community beneath *Acacia* canopies tends to be more diverse, palatable and nutrient-rich (Weltzin and Coughenour 1990, Coverdale et al. 2016), these results suggest that the strength of the associational refuge provided by spinescent neighbors is greater than the potential associational susceptibility conferred by growing near attractive neighbors (Barbosa et al. 2009); indeed, experimental removal of branches leads to a rapid increase in browsing damage (Coverdale et al. 2018), suggesting that the primary mechanism of this associational refuge is the physical inhibition of large herbivores by *Acacia*. Browsing damage on plants within treeless glades in contrast, was ~400% greater than in adjacent non-glade habitat (Fig. 2). Collectively, these results are consistent with previous reports of the role of physical defenses in intrinsic and extrinsic defense strategies in African savanna plant communities (Mcnaughton 1978, Cooper and Owen-Smith 1986, Louthan et al. 2014, Coverdale et al. 2016) and provide experimental evidence for the efficacy of physical defenses against large mammalian browsers in understory plants such as *S. campylacanthum*. Understanding the mechanism(s) of and interactions between intrinsic and extrinsic defenses, and particularly how they influence the distribution and abundance of species among refuge and non-refuge habitats, remains a promising area for future research.

Persistent differences in browsing intensity – driven, in this case, by proximity to spinescent overstory neighbors or to short-statured understory grasses – also appear to have exerted predictable effects on plant defense phenotype (Fig. 4). Associational resistance and associational susceptibility had strong, but opposing, effects on prickle density: associational resistance conferred by spiny trees decreased

prickle density by ~80%, whereas association with grasses within glades and clearings increased prickle density by ~45–60%. Taken together, these results indicate that intraspecific variation in defense phenotype can be quite large, even over relatively small spatial scales (e.g. 5–20 m² tree canopies) and time periods (≤3 years). These results further suggest that the identity and morphology of neighbors, which have previously been shown to affect defense phenotype in agricultural, boreal and model systems (Underwood et al. 2005, Kim 2017, Benevenuto et al. 2018), may be an important driver of such heterogeneity in savannas as well (Supplementary material Appendix 3 Fig. A4).

The observed influences of neighbors on defense phenotype could in principle be caused by several mechanisms. For example, competition with neighbors often decreases plant defense investment (Stamp et al. 2004, Donaldson et al. 2006), whereas volatile cues produced by damaged neighbors typically increase defenses through induced responses and/or defense priming (Farmer and Ryan 1990, Arimura et al. 2000). Neighbors may also affect
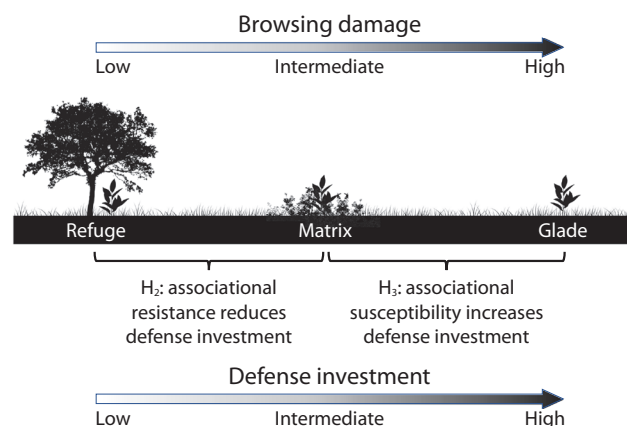

Figure 4. Schematic illustration of the effects of associational resistance and susceptibility on browsing damage and defense investment in *S. campylacanthum*. *Acacia* trees (left) provide associational refuges against large mammalian browsers. *S. campylacanthum* in grass-dominated clearings (right) suffer increased browsing damage relative to those in the matrix habitat outside glades (center).

defense phenotype by reducing the frequency or intensity of the herbivory cues that are necessary for induced responses, though such indirect mechanisms have received considerably less attention than those mediated by direct plant–plant interactions (but see Kim 2017, Benevenuto et al. 2018, Coverdale et al. 2018). Although the presence of trees is known to affect a variety of abiotic factors in savannas at the scale of individual canopies (Weltzin and Coughenour 1990, Belsky 1994), we found that experimental herbivore exclusion alone was sufficient to produce strikingly similar patterns of defense investment to those observed within and outside natural refuges (Fig. 2), suggesting that associational resistance, and not changes in abiotic conditions, likely drove observed patterns of defense phenotype. However, although abiotic conditions were comparable across experimental treatments at the onset of each large-scale manipulation (Augustine and McNaughton 2006, Goheen et al. 2013, Ford et al. 2014), we acknowledge the possibility that years of herbivore exclusion may have resulted in differences in some abiotic conditions between herbivore-exclusion and control plots. We attempted to minimize any such effects by selecting unassociated plants from comparable areas within and outside exclosure plots and by replicating all surveys across multiple plot pairs. We therefore believe that the primary difference between herbivore exclosure and control plots is the intensity of large mammalian browsing (Augustine and McNaughton 1998, Young and Okello 1998, Coverdale et al. 2016, 2018, Wigley et al. 2019).

Likewise, although soils and plants in glades are substantially enriched in N, P and micronutrients (Augustine and McNaughton 2006), our observations of defense investment by *S. campylacanthum* within experimental clearings – which are dominated by grasses and support greater densities of large mammalian herbivores but have not experienced major inputs of nutrients from livestock dung and urine (Ford et al. 2014) – suggest that differences in browsing damage are the primary driver of increased defense investment in open areas (Fig. 3). Plants within glades may experience associational susceptibility as a result of two mutually compatible mechanisms: 1) increased apparency to herbivores due to the dominance of close-cropped grasses (Castagneyrol et al. 2013), and/or 2) increased foraging intensity and/or encounter rate resulting from herbivore attraction to highly palatable neighbors (i.e. herbivore 'spill-over' sensu White and Whitham 2000). Collectively, the results of both exclosure experiments and experimental clearings indicate that the opposing indirect effects of associational resistance and susceptibility on focal plant defense phenotype arose from the contrasting effects of different neighbors on browsing herbivores, and are unrelated to spatial variation in resource availability in the form of light or soil nutrients (Fig. 4). Our results thus lend support to the growing body of evidence that variation in neighbor phenotype (whether intra- or interspecific; Underwood et al. 2014) can have marked effects on the diversity, phenotype and fitness of nearby plant (Hay 1986, Barbosa et al. 2009, Sato and Kudoh 2016, Kim 2017, Benevenuto et al. 2018, Coverdale et al. 2018).

The observed pattern of greater defense investment by more heavily browsed *S. campylacanthum* (Fig. 2A–B, 3A–B) may result from 1) selection for defended genotypes in high-risk areas (occurring over years or decades), and/or 2) induced responses to browsing damage (occurring over days to months). We suggest that there is little evidence for the former mechanism in this system, for several reasons. First, the timespan of our experimental exclosures and clearings is comparable to the lifespan of individual *S. campylacanthum* (Augustine and McNaughton 2006, Ford et al. 2014, Pringle et al. 2014). Additionally, we observed similar patterns of greater defense investment in the longer-lived tree *Acacia etbaica* on the margins of the same experimental clearings relative to adjacent uncleared areas (see also Ford et al. 2014). Together, these results indicate that changes in defense phenotype resulting from experimental manipulations can occur within a single generation for understory and overstory plants at this site, and we therefore consider rapid induction of defenses to be the likelier driver of defense heterogeneity. Moreover, induction (or relaxation) of physical defenses in response to increased (or decreased) browsing has been repeatedly demonstrated at our study site for both overstory and understory plants within experimental and natural refugia (Young 1987, Coverdale et al. 2018), and physical defenses are broadly inducible across species in African savannas (Wigley et al. 2019). We therefore suggest that induced responses to browsing may account for a sizeable proportion of the total observed variation in *S. campylacanthum* defense phenotype at our study site (Fig. 4, Supplementary material Appendix 3 Fig. A4). Similarly rapid induced responses to browsing in other members of the Solanaceae, including the congener *S. carolinense* (Kariyat et al. 2013), further support the inference that short-term plastic responses are a plausible driver of observed phenotypic variation in *S. campylacanthum*.

We quantified defense phenotype across sites that varied substantially in soil nutrient availability (e.g. glades versus experimental clearings) and found nearly identical levels of investment in carbon-based, physical defenses. Like other Solanaceae, *S. campylacanthum* also produces steroidal glycoalkaloids (a nitrogen-containing chemical defense) in fruits and leaves, yet little is known about interactions and tradeoffs between physical and chemical defenses in this (and other) species. Additional research would be required to determine whether associational effects have similar effects on chemical defenses in *S. campylacanthum* or, alternatively, whether resource constraints might cause chemical defenses to be down-regulated following the induction of prickles. How variation in resource availability relates to intraspecific variation in physical versus chemical defense investment has been poorly studied, and *Solanum* species may be especially valuable for future investigations of tradeoffs among defense traits.

Understanding the causes and consequences of variation in plant defenses has been a central goal in the study of plant–herbivore interactions (Coley et al. 1985, Burkepile and Parker 2017). Although many of the most prominent plant defense theories explicitly address interspecific variation

in defense phenotype (Stamp 2003, Hahn and Maron 2016), there is growing evidence that intraspecific variation in defense investment is widespread (Des Roches et al. 2017), may approach levels observed among species in plant communities or genera (Coverdale et al. 2018), and can impact the outcome of various ecological processes (Thorpe and Barbosa 1986). In African savannas, intraspecific variation in defense phenotype may have important implications for the persistence of plants in the face of intense top–down pressure by large mammalian herbivores: repeated browsing resulting from associational susceptibility drives a rapid accumulation of physical defenses, which in turn may reduce the proportion of plants vulnerable to herbivores. In contrast, plants within refuges invest little in intrinsic defenses, potentially allowing them to maximize fitness despite the constraints of competition with overstory neighbors.

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Supplementary material (available online as Appendix oik-06644 at <www.oikosjournal.org/appendix/oik-06644>). Appendix 1–3.