Manuscript submitted to *PNAS*

Biological Sciences: Evolution

Tradeoffs in the evolution of plant farming by ants

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Abstract [228 words]

Diverse forms of cultivation have evolved across the tree of life. Efficient farming requires that the farmer deciphers and actively promotes conditions that increase crop yield. For plant cultivation, this can include evaluating tradeoffs among light, nutrients, and protection against herbivores. It is not understood if, or how, non-human farmers evaluate local conditions to increase payoffs. Here, we address this question using an obligate mutualism between the ant *Philidris nagasau* and epiphytic plants in the genus *Squamellaria* that are farmed for their nesting sites and floral rewards. We focused on the ants' active fertilization of their crop and its protection against herbivory. We found that ants benefited from cultivating plants in full sun, receiving 7.5-fold more floral food rewards compared to shade-cultivated plants. The higher reward levels correlated with higher levels of crop protection provided by the ants. However, while high-light planting yielded the greatest immediate food rewards, sun-grown crops contained less nitrogen compared to shade-grown crops. This was due to lower nitrogen input from ants feeding on floral rewards instead of insect protein gained from predation. Despite this tradeoff, farming ants optimize crop yield by selectively planting their crops in full sun. Ancestral state reconstructions across this ant-plant clade show that a full sun farming strategy has existed for millions of years, suggesting that non-human farmers have evolved the means to evaluate and balance conflicting crop needs to their own benefit.

Significance [106 words]

In human cultivation systems, farmers increasingly use technology to gather data for evaluating tradeoffs between diverse - and sometimes conflicting - crop requirements to maximize yield. Some social insects have also evolved agricultural practices, but it is unknown how they evaluate local conditions to balance conflicting crop requirements. In the

obligate farming symbiosis between ants and plants in Fijian rainforests, we show how ant farmers also face key tradeoffs in crop cultivation. While ants cannot simultaneously maximize all services to their crops, our work demonstrates that they cultivate crops in high-light conditions to maximize floral food rewards, despite the nitrogen costs of this strategy. Evaluation of crop tradeoffs plays a key role in the evolution of farming strategies.

Introduction

Across the tree of life, organisms have evolved the ability to cultivate or 'farm' individuals of other species (1-6). This can involve habitual planting, husbandry, and harvesting of 'crops', such as seen in social amoebae rearing bacteria (1), marine snails propagating fungi (2), and damselfish cultivating seaweed (3). True agriculture, as defined by four key steps – namely habitual planting, cultivation, harvest and dependence of the farmer on the crop (7) - is restricted to social insects (ants, termites, beetles) cultivating fungi (7-10) and ants cultivating plants (11).

True agriculture is also practiced by humans, and generally involves the farmer deciphering and actively promoting the conditions that increase yield. For plant crops, this can involve evaluating tradeoffs among requirements for light, nutrients, and protection against herbivores. The ability to evaluate tradeoffs is important because farmers, both human and non-human, are unlikely to find conditions in which all crop needs are optimally and consistently met. For example, increasing nutrient conditions may inadvertently increase herbivore pressure (12), whereas increasing crop densities may negatively affect light conditions for individual plants (13).

While in human farming, technology and modeling are increasingly employed to evaluate complex tradeoffs (14, 15), it is not understood if, or how, non-human farmers evaluate local conditions to increase payoffs. There are cases of early insect agriculture in which tradeoffs are largely unresolved, and conflict can emerge between farmers selecting for edible hyphae, and fungal crops allocating resources to reproductive structures, such as mushrooms (16). Such conflicts, as seen between phylogenetically basal attine ants and their basidiomycete fungal crops, can result in low-productivity farming (16). In contrast, there are cases of agriculture involving a suite of adaptations and coevolved traits, such as practiced by leaf cutter or plant-farming ants, in which ant farmers appear to actively manipulate conditions to increase yield per unit input. Leafcutter ants, for example, mediate their local environment in ways that increase productivity, such as by sustaining large populations of Actinomycete bacteria in specific crypts that suppress parasites in fungal gardens (17). However, as farmers cannot simultaneously optimize all conditions (e.g. herbivore and pathogen defense, fertilization, modulating environmental variables), it is an open question as to whether, and how insect farmers mediate crop tradeoffs.

To answer this question, we studied an obligate farming mutualism between the ant *Philidris nagasau* (Dolichoderinae) and epiphytic plants in the genus *Squamellaria* (Hydnophytinae, Rubiaceae), occurring in Fijian rainforests on Taveuni and Vanua Levu islands (11). *Philidris*

nagasau workers cultivate multi-plant colonies of *Squamellaria* epiphytes that can contain 50 or more individuals whose plant-formed cavities in modified stems (domatia) house one queen and ~250,000 workers (11, 18). In this mutualism, the ants control dispersal, fertilization, and defense of the epiphytes. They do this by actively collecting *Squamellaria* seeds, planting them under the branch bark of their host tree, and subsequently protecting both seedlings and adults from herbivory (11, 18). The ants also fertilize these nutrient-limited (soilless) epiphytes by defecating on specialized and highly absorptive warts within a plant's tuberous domatium, which is also the ants' obligatory nest site (11, 18). In return, the ants feed on sugar and amino-acid-rich food rewards produced by the flowers' nectaries. The food rewards and the domatia, which contain a complex network of interconnected cavities (11, 18-21), are key to the ants' survival. Similar to the other 'true' agricultures in attine ants, termites, and ambrosia beetles (7), there is an obligate dependence of the farmer on the crop (11, 18). *Squamellaria* crops are likewise obligately dependent on the farmer (11, 18).

By controlling dispersal, fertilization, and defense of the crop, the farmer could potentially maximize crop productivity by modulating growing conditions, but this remains poorly understood. Specifically, it is unknown if farming strategies are tailored to particular environmental conditions. Because of the challenges in empirically manipulating most farming mutualisms, it is difficult to test how demands for light, nutrients, and protection are evaluated by insect farmers. Here, we address this challenge by studying the *Philidris nagasau*-*Squamellaria* farming mutualism under different natural light conditions. Our aim is to test if ants optimize farming and defense of their crop to match local environments. Using productivity metrics, behavioral assays, and stable isotope analyses, we asked: (i) whether crop productivity (i.e. food rewards) is influenced by light conditions, (ii) how defense levels provided by farmers to their crop differed along light gradients? and (iii) whether there are tradeoffs between farming traits, such as active fertilization, and other crop services across light levels? We also traced the evolutionary history of farming strategies in the *Philidris*/*Squamellaria* mutualism, using a densely-sampled plant phylogeny, to ask if light niche is correlated over evolutionary time to defense and fertilization of the crop by the ants.

Results and discussion

Crop productivity varies along a light gradient and correlates with herbivore defense levels

First, we asked how crop productivity, as measured by food rewards to ants, varied along a natural light gradient. Mature *Squamellaria* flowers provide *P. nagasau* workers with a sugary sap rich in sucrose and amino acids that is only accessible to this ant species (21). We quantified food reward (number of post-anthetic nectaries, ref. 21) on $N = 133$ shoots belonging to 50 *S. wilsonii* plants spanning a light gradient in Taveuni rainforest canopies categorized as full shade, mid light exposed, and full sun, controlling for shoot size (*Materials and Methods*).

We found that food rewards were 7.5-fold higher in plants cultivated in full sun than in plants cultivated in full shade (**Fig. 1A**): higher light levels consistently led to more flowers and hence food availability (**Fig. 1A**; ANOVA: $F_{2,150} = 20.19$, $P < 0.001$) (Tukey's HSD post hoc test: full sun | full shade, $P = 0.0$; mid exposed | full shade, $P = 0.003$; mid exposed | full sun, $P = 0.0004$). These data confirm that crop yield varies as a function of light regime and is maximized when the crop is grown under high light conditions.

We then analyzed the links between crop yield and ant defense, asking how defense levels provided by farmers to their crop, differed along light gradients. Specifically, we tested whether higher rewards were correlated with increased crop protection, as this has the potential to generate positive feedback for cultivation in sun conditions. We quantified herbivory under different light levels and found that < 25% of the leaves of plants cultivated in full sun showed herbivore damage. In contrast, herbivory increased to nearly 90% in plants cultivated in full shade (**Fig. 1B**; ANOVA: $F_{2,150} = 70.05$, $P < 0.001$) (Tukey's HSD post hoc test: full sun | full shade, $P = 0.0$; mid exposed | full shade, $P = 0.0$; mid exposed | full sun, $P < 0.001$). The anti-herbivore defense role of *Philidris nagasau* ants has also been shown in previous ant-exclusion experiments (18).

Lower herbivory levels in full-sun could either be the result of higher ant defense or fewer herbivores. We therefore measured ant patrolling behavior across light conditions, systematically recording the distribution of *P. nagasau* workers on plant surfaces (*Materials and Methods*). We found significantly more patrolling ants on full-sun plants compared to fully shaded plants (**Fig. 1C**, t-test: t = -16.17, df = 14.08, $P < 0.001$), with a significant negative correlation between the levels of rewards and herbivory along the light gradient (**Fig. 1D**; $F_{1,151}$=105.2, P <0.001; Pearson correlation coefficient = -0.64). This suggests that ants are more likely to patrol and defend *Squamellaria* under full-sun conditions when plants bear more flowers, and more food rewards are available. This can also help generate partner-fidelity feedbacks (18), such that there is alignment of colony size with plant size.

We further tested the alternative explanation that there are fewer herbivores in full sun by running two additional experiments to determine herbivory levels across our three light environments. While studies of herbivory in forest canopies generally find higher densities of herbivores under sun compared to shade conditions, this can vary locally (22, 23). Therefore, we quantified herbivory in three representative plant species (*Macaranga harveyana*, *Miconia calvescens*, *Psychotria* sp.) with branches placed in experimental blocks across different light levels (Experiment 1). After eight days, we imaged all leaves and calculated herbivory levels (*Materials and Methods*). Our GLMM analysis revealed no consistent association between herbivory and light level, except in *Macaranga* where we found a strong positive effect of light environment ($F = 5.6$, $P = 0.01$), with a trend toward more herbivory in full sun ($t = 1.96$, $P = 0.056$) (*SI Appendix*, **Fig. S1**, **Table S1**). We then repeated this experiment with *Squamellaria wilsonii* (Experiment 2), and again found no significant association between herbivory and light level (*SI Appendix*, **Fig. S2, Table S2**). Taken together, these data suggest that the correlation between higher food rewards and lower

herbivory in full sun-grown *Squamellaria* is a result of increased patrolling by ants, not lower herbivore abundance.

The tradeoff between nitrogen fertilization and defense by ants along a light gradient

In the variables quantified thus far – (i) food rewards to ant farmers and (ii) defense by ants against herbivores – the interests of the farmer and its crop are aligned in full sun conditions: ants are offered more rewards and plants benefit from better protection. However, ants perform another crucial farming function, namely fertilizer input. This function is especially important for nutrient-limited epiphytes that grow in soilless conditions. Targeted fertilization is a key trait in this farming mutualism and involves *P. nagasau* workers defecating exclusively on highly absorptive warts on the inner walls of domatia (18). This active fertilization continues from the seedling to the adult stage of *Squamellaria* plants (11, 18). However, it is unknown whether there are tradeoffs between active fertilization and other ant-mediated crop demands across light levels.

To evaluate levels of nitrogen fertilization by *P. nagasau* under different light conditions, we collected tissue from four *S. wilsonii* individuals from each of the three light environments. We sampled six tissues (leaves, stem, roots, smooth cavity tissue, warty cavity tissue, and tuber tissue, see *SI Appendix*, **Fig. S3A** for description) and quantified nitrogen content in different tissues along the light gradient. For each tissue, we sampled triplicates, leading to a total of $N = 18$ samples per plant, and a total of $N = 216$ samples.

Given the well-established positive relationship between leaf nitrogen and photosynthetic capacity (24, 25), we had expected to find higher N levels in plants cultivated under sun conditions. However, we found the opposite: across all tissues tested, plants growing under full sun contained the least amount of N per g tissue (*SI Appendix*, **Fig. 2, Fig. S3B**, GLMM, $t = -2.58$, $P = 0.03$), with N content increasing under mid- and fully-shaded conditions. We found that the difference in N content between sun and shade was particularly pronounced in the warty cavity wall (**Fig. 2**), the location in the domatia where ants defecate to fertilize their crop. Here, we found that warty tissue of sun-grown plants had the lowest N per g of tissue.

The low N content of full sun-cultivated plants could arise from two scenarios. First, if *Squamellaria* plants grow faster in full sunlight, this could lead to lower N level by diluting nitrogen inputs. However, past work argues against this possibility. Specifically, a tight linear correlation has been found between the size of the worker ant colony and the size of *Squamellaria* domatium (i.e. a measure of plant growth) (18). This means faster plant growth is mirrored by proportional increase in ant workers, and hence nitrogen inputs, thus preventing a dilution effect. We verified that this relationship between worker population and domatium size holds across light levels (*SI Appendix*, **Fig. S4**). This suggests that faster growth in full sun is matched by a proportional increase in fertilizing ants, preventing N dilution.

A second scenario is that nitrogen input is mediated by ant diet and differs across light levels. Under this scenario, an ants' feces will reflect its diet, which will differ under shade versus sun conditions. This is because plants cultivated by ants in the shade produce fewer flowers and hence provide less food compared to sun-grown plants (**Fig. 1A**). As a result, *P. nagasau* workers may be forced to forage for supplementary sugar and protein elsewhere in the canopy, including foraging as predators for insect prey. If a greater proportion of the shade farmer's diet consists of insects, rather than crop-produced rewards, this will lead to a greater external N input into the shade system.

To test this explanation, we traced back the nitrogen in plant tissues to either plant-derived resources or insect-predation using N stable isotope ratios ($\delta^{15}N$), following previous studies (22, 23). We collected *P. nagasau* from $N = 8$ colonies across the light gradient and sampled the warty-walled tissue of their domatia where ant defecation takes place. We found a tight positive correlation (Pearson's correlation coefficient = 0.86; $P = 0.005$; *SI Appendix*, **Fig. S5**) between ant $\delta^{15}N$ and warty-walled tissue N content. This correlation implies that light-dependent food availability (from flowers or insects) drives the higher nitrogen input in shade conditions.

We then quantified hunting behavior. If higher N levels in low light environments comes from more insect hunting, we should observe different *P. nagasau* worker behavior in shade vs. light colonies. By monitoring workers outside their crops in full-sun vs. shaded colonies, we found significantly higher insect hunting activities in the shade, compared to sun colonies (*SI Appendix*, **Fig. S6A-C**). To further test the ant's hunting behavior in sun vs. shade environments, we offered a cockroach placed one meter from a *Squamellaria* crop (*Materials and Methods*). We found that both the time of discovery, and the time of arrival of the second recruit was significantly shorter in the shade (*SI Appendix*, **Fig. S6D-F**). This suggests that in sun conditions, these canopy epiphytes are forced to more tightly recycle nitrogen because of a lack of external N inputs. In contrast, shade-grown plants receive more external N from ant feces because ants are forced to forage for insects to supplement their food.

A second test of the hypothesis that light influences fertilization input by ants via food rewards is to compare N levels of *Squamellaria* without food rewards in the sun and shade conditions. To do so, we took advantage of *Squamellaria grayi*, a species that has lost the ability to produce food bodies over evolutionary time (20, 21). We compared nitrogen levels of both ant workers and warty tissue grown in shade vs. sun in *S. grayi*. In contrast to *S. wilsonii* that does produce food bodies, we found there was no significant difference in N levels in shade vs. sun conditions in *S. grayi* that does not produce food rewards (*SI Appendix*, **Fig. S7**), further supporting our idea that plant food rewards influence ant N input across a light gradient.

We tested a final alternative explanation for the lower N content of sun-exposed *S. wilsonii*, namely that they might be facultative (inducible) CAM plants. CAM plants have a substantially lower rubisco content, hence contain less nitrogen per g of tissue. Under this scenario, *S. wilsonii* would switch on CAM metabolism under full sun, leading to lower

nitrogen levels. Three of the six Fijian *Squamellaria* farmed by *P. nagasau* indeed have CAM capabilities (20), but when we tested *S. wilsonii* for CAM activity by measuring the $\delta^{13}C$ ratio in warty tissue, roots and leaf samples across the light gradient all samples showed values near -30‰ (*SI Appendix*, **Fig. S8**), ruling out substantial CAM activity in this species. This is also supported by leaf morphology: while the three CAM *Squamellaria* all have succulent leaves, *S. wilsonii* has thin leaves (20).

Together these experiments show that tradeoffs exist between two key services offered by the ant to the plant, namely defense, which is highest under full sun, and ant-mediated nutrition, which is highest in the shade. By populating a probabilistic model with our empirical data, we find a clear tradeoff between defense and nutrition (**Fig. 3**). Similar to tradeoffs faced by human farmers, ants cannot simultaneously maximize all services to their crops (**Figs. 1-3**). Given that food rewards are likely to yield the highest immediate individual benefit to the ants, it is expected that ants will plant crops in full sun (**Fig. 1**), even if this results in lower fertilizer inputs to their crop.

Farming tradeoff between defense and nutrition over space and time

The identification of a light-mediated tradeoff between defense and nutrition prompted us to investigate the extent to which farming ants can manipulate their crop's light environment over space and time. *Squamellaria* species are either dispersed by birds (non-farmed) or by *P. nagasau* ants (farmed) (11). We therefore asked if there was a difference in the height at which farmed vs. a non-farmed *Squamellaria* species were found when they co-occurred in the same forest. We found that farmed species were significantly higher in the canopy compared to non-farmed species, dispersed by birds (N = 30, *SI Appendix*, **Fig. S9,** t-test: $t = 7.57$, $df = 45.44$, P-value < 0.0001**)**. We then determined the mean light niche for the nine Fijian *Squamellaria* species, and found that farmed species are significantly more sun-exposed than non-farmed species (**Fig. 4B**; Fisher's exact test (farmed vs. non-farmed): $\chi^2 = 67.79$, $df = 2$, $P < 0.001$). In farmed *Squamellaria*, seedlings were found preferentially on tree trunks that received direct sunlight, suggesting that light is a potential cue for where ants plant *Squamellaria* seeds.

Given the evidence that farmed *Squamellaria* is associated with sun-exposed sites, why is there any diversity in the height at which ants cultivate their crops? Two hypothetical strategies could be employed: (i) a *balanced cultivation* in which *Squamellaria* are planted at various light levels, ensuring a balance of high crop productivity in sun-exposed plants, and high nitrogen inputs in shade-exposed crops. This would be effective since ant workers move among domatia (Supplementary figure S2a in ref. 11); (ii) an *immediate rewards-first cultivation* strategy, in which *Squamellaria* are consistently planted in sun-exposed sites only. If this hypothesis is true, *Squamellaria* farmed within the same ant colony will be predominantly found in a single light level.

To test which strategy is more likely, we mapped the light niche of 657 *Squamellaria wilsonii* individuals from $N = 100$ *Philidris nagasau* colonies in Taveuni (*Materials and Methods*).

We found that light level, while controlling for tree size, was a predictor of crop number per colony size, with shaded colonies being significantly smaller than sun-exposed colonies (**Fig. 4A**; ANOVA, $F_{4,95} = 5.82$, $P < 0.001$). Specifically, of the 657 *S. wilsonii*, 406 were growing in full sun, 208 were growing in mid-exposed conditions and 43 grew in a fully shaded environment (**Fig. 4A**). We found 69% of the colonies occurred at a single light level, with 22 colonies farming full sun and mid-exposed crops and only 9 colonies farming mid-exposed and fully shaded crops (**Fig. 4A**). In the cases where colonies spanned two light niches, this was generally associated with host tree growth, namely *Squamellaria* crops becoming shaded by the growth of large branches of the host tree. However, we did observe rare cases of fully-shaded *Squamellaria* (including seedlings in a few instances), growing low in the canopy, which reflects that farming ants have retained the ability to plant *Squamellaria* outside full sun. More generally, these data reveal the absence of the *balanced cultivation* strategy and are more in line with ants maximizing high-light productivity as posited by the *immediate rewards-first* hypothesis. These data also suggest there are few costs associated with a mostly plant-based diet for ants versus an insect-protein rich diet in shade conditions, consistent with the documented herbivorous diets of arboreal ants (26, 27). This work suggests more research is needed in the role of gut microorganisms in the nutrition of the ants in shade vs. sun conditions.

Evolutionary history of farming traits

Given the evidence that the farmer controls the light environment in a way that maximizes crop productivity, we next asked how plant traits related to farming have changed across the Hydnophytinae, the ant-plant clade to which the genus *Squamellaria* belongs. We traced the evolution of three farming-related traits on the Hydnophytinae phylogeny (28), which contains obligately farmed species, facultatively farmed species, generalist ant-associated (but non-farmed) plants, and non-ant associated plants. Specifically, we focused on the (i) presence of food rewards; (ii) domatium wall differentiation, as a measure of the emergence of specialized wart structures to capture ant-derived fertilizer (18); and (iii) location in the canopy as a measure of the species' light niche (*Materials and Methods*).

We found evidence that farmers have influenced their crop's growing niche over evolutionary time. Specifically, high light niches have evolved four times, matching independent colonization by *Philidris* farming ants (**Fig. 4C**). Tests of correlated trait change using Pagel's (29) approach strongly supported the correlated evolution of food rewards (nectaries of the specific type found in five out of six farmed *Squamellaria*; ref. 21) and domatium wall differentiation (**Fig. 4C**; $BF_{[independent\text{-}dependent]} = 72.53$; *SI Appendix*, **Fig. S10**). Similarly, shifts to high-light environments were correlated with the evolution of the food rewards and domatium wall differentiation (**Fig. 4C**; $BF_{[independent\text{-}dependent]} = 34.12$; *SI Appendix*, **Fig. S11**; $BF_{[independent\text{-}dependent]} = 73.16$; *SI Appendix*, **Fig. S12**). This suggests that traits linked to nutrition and defense evolved jointly.

We next compared the transition rates under the best-fitting correlated evolution model to ask two questions. First, are food rewards more readily gained in variable light (*q12*) or in high-light environments (*q43*)? And second, does domatium wall differentiation evolve more

readily in variable-light environments (*q12*) or in high-light environments (*q43*)? We found that both food rewards and domatium wall differentiation evolved more readily in canopy species, associated with high light conditions (Food rewards [mean ± SE]: *q12* = 0.01± 0.0003; *q34* = 3.05 ± 0.09, Kolmogorov-Smirnov test: D = 0.979, $P < 0.001$; Wall differentiation: *q12* = 0.01 ± 0.0003; *q34* = 13.95 ± 0.0769; Kolmogorov-Smirnov test: D = 1, $P < 0.001$; **Figs. S11-S12**). This shows that farming traits evolved in a high light niche, and suggests that despite a reduction of nitrogen to their crops, selection has favored the planting of crops in high light conditions.

Conclusion

Human farmers balance diverse crop requirements across different environments to optimize yield. Our data revealed that *Philidris nagasau* farmers likewise optimize crop yield by controlling their crop's light environment. This farming strategy has evolved despite lower nitrogen inputs associated with sun conditions because it most directly benefits farmers. Further, nitrogen input from farmers cultivating crops in full sun is still roughly three times higher than any by-product fertilization found in non-farmed *Squamellaria* species (18), suggesting that the loss of nitrogen in full sun may be trivial in the short term.

More generally, our work highlights the role of tradeoffs in the evolution of insect agriculture. While human agriculture has seen an increase in the use of technology to gather data for evaluating tradeoffs, the ant-plant farming mutualism has been subject to millions of years of natural selection. While ants cannot simultaneously maximize all services to their epiphyte crops, our work supports the idea that they buffer environmental variation by selecting the environment where their crop is most productive.

Materials and Methods

Study sites

In September 2014, March 2015, August 2016, August 2017, July 2018, and June 2019 the first author conducted fieldwork on Viti Levu, Vanua Levu, and Taveuni, Fiji. In July-August 2017, G.C. and E.T.K. jointly conducted fieldwork in Taveuni. The study sites in Viti Levu were Colo-i-Suva forest reserve in the south of the island (S 18° 1' 46.808", E 178° 24' 0.4175") and forest around Navai in the center of the island (S 17° 37' 49.5979", E 177° 58' 34.9315"). In Vanua Levu, the study sites were in the Waisali forest reserve (S 16 38'19.8", E 179 13'19.7"), and along the Cross Island road before the bifurcation to Nabouwalu and Labasa. In Taveuni, the study sites were along the trail to DesVoeux peak and Mt. Manuca on the western side of the island (S 16° 48' 25.8133", E 179° 56' 36.6843"), and at the end of Lavena coastal walk, Bouma heritage park, on the eastern side of the island (S 16° 51' 45.4433", E 179° 54' 6.5149").

Defining the light gradient of farming and non-farming ant/plant symbioses in Fiji

Our study system consisted of a recently described farming mutualism involving the ant species *Philidris nagasau* (Dolichoderinae) which obligately and exclusively cultivates

Squamellaria ant plants (11). *Squamellaria* taxonomy follows Chomicki and Renner (20). *Squamellaria wilsonii* is the most abundant species on Taveuni (11).

Initially, we aimed to provide a continuous measurement of the light level associated with each plant. However, this metric proved to be unreliable due to (i) frequent cloud shading, (ii) wind patterns moving branches above the *Squamellaria*, and (iii) daytime, which all drastically affected light level readings. We thus opted for a discrete categorization of a light environment based on canopy shading. We classified the light environment as (i) *fully shaded*, when 90-100% of canopy cover over-shaded the ant plants – an environment typical of the rainforest under story; (ii) *mid-exposed*, when 40-75% of the canopy above a *Squamellaria* plant was shaded; (iii) *fully exposed*, when a maximum of 20% canopy cover shaded the *Squamellaria* plants, an environment in which they were exposed to direct sunlight for the majority of the day. We used this same categorization for all Fijian *Squamellaria* species and counted the number of individuals in fully shade, mid-exposed, and fully exposed environments.

Quantifying herbivory along a light gradient

Our aim was to compare defense and fertilization services provided by *P. nagasau* to *S. wilsonii* plants, and the rewards provided by *S. wilsonii* to *P. nagasau* across a light gradient. Except in cases where *Squamellaria* plants were accessible near the ground, we accessed the plants by tree climbing, using a rope secured by a partner on the ground. This technique allowed long stays in the canopy with minimal disturbance of ant colonies. To measure the effectiveness of the defense function of *P. nagasau* mutualists, we quantified leaf herbivory in 50 *S. wilsonii* plant individuals spanning the light gradient (10 fully shaded, 22 mid-exposed and 18 fully exposed). For each individual, we used three shoots (more when possible, and only two in one instance), leading to a total sample size of $N = 30$ for fully shaded, $N = 71$ for mid-exposed and $N = 53$ for fully exposed plants. Leaf herbivory was measured as the percentage of leaves eaten at more than 10%. We also confirmed in the field that using a different threshold (i.e. 50%) did not change our results:

$$Herbivory = \frac{Number\ of\ leaves\ eaten\ at\ >10\%}{Total\ number\ of\ leaves\ per\ shoot} * 100 \quad (Eq.\ 1).$$

Quantifying plant-produced food rewards along the light gradient

Using the same 50 *S. wilsonii* individuals ($N = 153$ shoots spanning the light gradient), we quantified the food reward provided by the crop. These rewards are only accessible to the *P. nagasau* mutualists (21). We counted the number of post-anthetic nectaries and divided this number by the number of internodes of the shoot. The number of internode per shoot has previously been shown to directly reflect the age of the *Squamellaria* shoot and thus allows to compare the food rewards per shoots regardless of shoot age (11, 21).

Quantifying ant patrolling activity on crops along the light gradient

To test whether lower herbivory levels in full-sun were the result of higher ant defense rather than fewer herbivores, we measured ant patrolling across light conditions by recording the distribution of *P. nagasau* workers on the plant surface. The expectation was that if the lower

herbivory level of full sun *Squamellaria* reflected more ant defense, we should observe a difference in patrolling activity in sun vs. shade *Squamellaria*. Patrolling activity was measured as the percentage of ants present on shoot (leaves, stem, food rewards [where most ants are found; cf. ref. 21]) versus the domatium surface. We selected $N = 8$ *Squamellaria wilsonii* from distinct *Philidris nagasau* colonies growing in full shade, $N = 13$ growing in mid-exposition, and $N = 20$ growing in full sun. We recorded the number of ants on the shoots versus the domatium surface for 5 consecutive minutes on each plant. Observations were made on sunny days at 11.00-12.00 AM and 1.00-2.00 PM, and late morning and early afternoon observations were averaged.

Testing the effect of the light level on herbivory

To experimentally test the effect of light level on leaf herbivory, we designed an experiment (experiment 1) wherein we placed shoots from three species in 15 blocks of cut plastic bottles filled with water (*SI Appendix*, **Fig. S1A**). This experiment was performed in the rainforest of Taveuni island, along the road leading to DesVoeux peak. We selected plants from three families: *Macaranga harveyana* (Euphorbiaceae), the Neotropical invasive *Miconia calvescens* (Melastomataceae), and *Psychotria* sp. (Rubiaceae) that likely differed in leaf chemistry. *Macaranga* and *Miconia* were growing in full shade, while *Psychotria* plants were sun exposed. In all cases, twigs used in the experiments came from the same plant individual to control for intra-specific variation in leaf chemistry. We ensured that each twig had leaves of comparable physiological age by using twigs with similar number of leaves counting from the apex. We created $N = 5$ blocks per light environment (full shade, mid-exposed, and fully exposed) each with three twigs per each of the three species (hence nine twigs per bottle).

We selected twigs free of herbivory or where leaf herbivory was <10% on any leaf (removing some damaged leaves while selecting the twigs, prior to the experiment). The experiment lasted 7 days. On the 8th day, all twigs were collected, and leaves were removed one by one (total $N = 685$ leaves), and photographed. We scored all leaves eaten at >10%, and calculated herbivory as above (Eq. 1). In June 2019, we replicated this experiment but this time only using sun-grown *S. wilsonii* twigs (experiment 2; same field site). We collected 15 twigs from a single sun-exposed *S. wilsonii*, and placed each twig in a cut bottle, using $N = 5$ blocks in our three light levels as above. This allowed to directly test the alternative hypothesis that light-dependent background herbivory rates could explain our results.

For the statistics, we used a generalized linear mixed model using the function 'lmer' implemented in the R package 'lme4' (30). We set herbivory as dependent variable, and light, species (for experiment 1), and leaf number as fixed-effect predictors, with leaf number nested within species (to represent the structure of each experimental block). We used 'block' as our random effect predictor, and again we modelled the leaf number per species nested within the block. Model estimates are shown in *SI Appendix* Table S1 and Table S2.

Quantifying nitrogen fertilization along the light gradient

Fertilization by defecation is a key function in this farming mutualisms and involves *P. nagasau* workers defecating exclusively on highly absorptive warts on the inner domatium

walls (11, 18). To evaluate the nitrogen fertilization by *P. nagasau* in the different light conditions, we collected four *S. wilsonii* individuals for each light environment. We next sampled six plant parts (leaves, stem, roots, smooth cavity tissue, warty cavity tissue, and tuber tissue, see *SI Appendix*, **Fig. S3A** for description). For each tissue, we sampled triplicates, leading to a total of N =18 samples per plant, and a total of N =216 samples. Samples consisted of tissue aliquots that were silica-dried in the field and analyzed via isotope ratio mass spectrometry.

Quantifying nitrogen content in ants and *Squamellaria* when food rewards for the ants are absent

To confirm that light influences fertilization input by ants, we compared the nitrogen content of *Squamellaria* that do not offer food rewards to ant farmers in the sun vs. shade. We took advantage of *Squamellaria grayi*, a farmed species that has lost the ability to produce food bodies over evolutionary time (20, 21). The expectation was that if the light-dependent nitrogen input derives from the abundance of food rewards, absence of food rewards should lead to similar nitrogen levels in both ant workers and plant tissues. We thus sampled *Philidris nagasau* workers and *Squamellaria grayi* plant tissues from N = 5 colonies from full sun vs. N = 5 colonies in full shade environments. Ant workers and plant tissues (leaves, stem, roots, smooth cavity tissue, warty cavity tissue, and tuber tissue) were sampled and dried as above, and submitted to isotope ratio mass spectrometry.

Isotope Ratio Mass Spectrometry

For measurements of nitrogen content, $\delta\,^{15}N$ ‰ and $\delta\,^{13}C$ ‰ measurements, plant tissues were harvested and dried with silica gel. Samples were ground to fine powder with a mixer mill MM301 (Retsch). Stable N and C isotope values relative to air N_2 and to the Pee Dee belemnite standard, respectively, were determined from approximately 10 mg (± 0.5 mg) of sample in case of N isotopes and from 200 µg of sample in case of C isotopes with the stable isotope ratio mass spectrometer MAT253 (Thermo Scientific, Wilmington, USA), the organic elemental analyzer Flash 2000 Elemental Analyzer (Thermo Scientific), and a Conflo IV (Thermo Scientific) at the Institut für Geowissenschaften (Institute for Geological Sciences) at the University of Mainz, Germany.

Monitoring ant hunting behavior in full sun vs. in the shade

Our hypothesis that farming ants provide more nitrogen in the shade implies more hunting activity in the shade as opposed to the sun. To quantify this, we monitored hunting behavior in N = 5 *Philidris nagasau* colonies living in *S. wilsonii* growing in full sun and N = 5 plants growing in the shade, both along the DesVoeux peak track, Taveuni. We monitored hunting behavior in a radius of 2 meters from each *Squamellaria* individual, and in each case, we selected *Squamellaria* of similar sizes in shade versus sun. To ensure replicability in our measure of hunting behavior across colonies, we recorded hunting behavior for 10 minutes between 12.00 AM – 1.00 PM on sunny days (*SI Appendix*, **Fig. S6A**). Next, we performed an experiment to determine the times to discovery and to arrival of the first recruit (second ant) to an immobilized cockroach placed in the full shade or full sun at 1 m of a *S. wilsonii*. The experiment was replicated 5 times in each light environment (*SI Appendix*, **Fig. S6D-F**).

Determining the light niches of farmed vs. non-farmed *Squamellaria*

We quantified distribution patterns of $N = 30$ *Squamellaria imberbis* (farmed) and the same number of *S. wilkinsonii* (bird dispersed) in a region where both coexist in a rainforest on the island of Vanua Levu around the Waisali forest reserve (S 16 38'19.8", E 179 13'19.7"). We determined the height at which *Squamellaria* were growing using the standard tangent method (31). Knowing the distance angles between the horizon and the *Squamellaria* at two adjacent distances next to the tree, one obtains:

$$H = \frac{D \cdot \tan(\alpha) \cdot \tan(\beta)}{\tan(\alpha) - \tan(\beta)}$$

Where H is the height of the *Squamellaria* in the tree, D, the distance on the ground from which the two angles were measured, α, the angle between the horizon and the *Squamellaria* from the point closer to the tree, and β the angle from the more distant point on the ground. For each of the two species, we measured $N = 15$ specimens along the same transect.

Next, we determined the light niche of all nine *Squamellaria* species, which grow in forests with different height. We quantified the proportion of plants per species growing in full sun, mid-exposed or full shade along transects across Viti Levu, Vanua Levu and Taveuni islands in Fiji (see *Field Sites*). Sample sizes were as follow: *S. thekii* ($N = 20$), *S. grayi* ($N = 40$), *S. huxleyana* ($N = 20$), *S. major* ($N = 20$), *S. imberbis* ($N = 100$), *S. wilsonii* ($N = 100$), *S. tenuiflora* ($N = 60$), *S. wilkinsonii* ($N = 60$), *S. jebbiana* ($N = 20$).

Quantifying the light niche of *Squamellaria wilsonii*

To quantify the light niche of *Squamellaria wilsonii* and to address whether *Philidris nagasau* ants plant across light levels, ensuring a balance of high crop productivity in sun-exposed plants, and high nitrogen inputs in shade-exposed crops ('balanced cultivation'), or whether a single colony would consistently plant *Squamellaria* in sun-exposed sites ('immediate-rewards-first cultivation strategy'), we mapped the light niche of 657 *Squamellaria wilsonii* individuals from $N = 100$ *Philidris nagasau* colonies in Taveuni, along the DesVoeux peak track as well as near the hydroelectric Somosomo dam. For each of the 100 *Philidris nagasau* colonies, the *Squamellaria* individuals occupied by the respective ant colonies (excluding seedlings that could not be accurately quantified) were counted, and the light level (full shade, mid-exposed, full sun) of each was determined, following our classification scheme described above. We then plotted the percentage of plants in each of the light levels for the 100 colonies.

Statistical analysis

We compared (*i*) plant defense by ants in the different light environments, (*ii*) ant-mediated nitrogen fertilization in the different light environments, (*iii*) food reward abundance in function of the light level, and (*iv*) the correlation between food reward abundance and herbivory. For (*i*, *ii*, and *iii*), we used first one-way ANOVA followed by post-hoc tests when the data were normally distributed (as verified by a Shapiro-Wilk test) and when there were no random effect to take into account. When data were non-normal and/or random effects were present, we used a generalized linear mixed model (GLMM). Specifically, we used a

GLMM to test the effect of light environment on nitrogen level. We used a GLMM approach modelled under penalized quasi-likelihood (PQL), using a Gaussian probability with the function 'glmmPQL' implemented in the R package MASS (32). In this model, light level and tissues are the fixed effects while specimens (from which several samples are drawn) are the random effect.

We further used multinominal logistic regressions to obtain a probabilistic framework of plant defense by ants, ant-mediated nitrogen fertilization, and food reward abundance in function of the light environment categories (**Fig. 3**). For all logistic regressions, we first used the actual overall sample size (N =153 for food rewards and plant defense and N =205 nitrogen fertilization), and we subsequently used a simulated sample size of 1,000. In all cases, results between real and simulated sample sizes were comparable. For the multinomial logistic regressions, we used the R package 'MASS' (32), and dependencies, relying on the function 'polr'. To test the correlation between food reward abundance and herbivory, we used Pearson's correlation coefficients implemented in function 'corr' of the 'stats' package in the R base. We used the 'stats' package in the R base and 'anova' from the car package to generate *P*-values, and the package 'ggplot2' (33) for plotting graphs, all in R version 3.4.3.

Testing the evolutionary correlation of defense, nutrition, and niche evolution

We inferred the evolutionary history of defense and nutrition traits on the Hydnophytinae phylogeny (28) and then tested for their correlated evolution. We coded all 76 ingroup species for the absence (coded '0') or presence (coded '1') of concealed post-anthetic flower rewards (21, 28) as defense trait (*this study*). The majority of Hydnophytinae form nutritional symbioses with ants, either involving specialized Dolichoderinae species (from the genera *Philidris* and *Anonychomyrma*) or involving generalist species (11, 18-21, 28). A key nutrition trait is the structure of the inner domatium walls. Specialized species have domatia with differentiated warty tissue where ants defecate and non-absorptive smooth tissue where they raise their brood. By contrast, species forming generalist symbioses have poorly differentiated domatium walls with 'wart-like' and 'smooth-like' areas that are variable over space and time. Species that have lost symbioses with ants have lost the absorptive warts (18). In order to perform a test of correlated evolution, we coded domatium internal wall type as a binary variable with either poorly differentiated walls or wart absence as '0' and differentiated walls as '1'.

We next studied the evolutionary history of light niche in the Hydnophytinae. Hydnophytinae that form facultative symbiosis with many generalist ant species are predominantly found in the lowlands, non-mutualists occur typically at high altitude, and specialized species are found across different altitudes (28). We coded the light niche as a binary trait (light-variable coded '0' or high-light coded '1') based on literature (28, 34-37). Light-variable environments correspond to species that can be found at widely varying height in the canopy, and which typically are species dispersed by birds and forming generalist symbioses with various ant species (11). We included in this category species that are the few *Myrmecodia* species inhabited by lowland rainforest *Anomychomyrma* ants, which grow in shaded light environments. We categorized species of high light environments by their reported ability to

live high up in the rainforest canopy, in open, semi-dry forests, or even as terrestrial, soil-rooted plants in open highlands in New Guinea. In all three traits, 0 → 1 transitions correspond to the evolution of defense, nutrition traits or high light niche.

To infer the evolutionary history of defense and nutrition traits, we used two approaches. First, a stochastic mapping method, relying on the function 'make.simmap' in the phytools package (v. 04-60) (38), which implements the stochastic character mapping approach developed by Bollback (39). We estimated ancestral states under the best-fitting model as determined by AIC and then simulated 1,000 character histories on the MCC tree. We summarized the 1,000 simulated character histories with probability density plotted on the phylogeny using the function 'densityMap'.

Second, we used the Bayesian reversible jump MCMC approach implemented in BayesTraits v. 2 (40), using a sample of 1,000 trees from the BEAST analysis to account for phylogenetic uncertainty, a chain of 50 million generations with rate coefficients and ancestral states sampled every 1,000th generation. We ensured that the acceptance rate was between 20 and 40%, as recommended in the manual, and reconstructed the nodes of interest using the command 'addnode'. We reconstructed all key nodes and reported the probabilities above nodes in Fig. 4.

To test for possible evolutionary contingencies or correlations between defense and nutrition traits and the niche type, we used a method for discrete traits (29, 40) as implemented in BayesTraits v. 2 (40). We again used the maximum clade credibility (MCC) tree from BEAST but pruned the outgroups and first ran a model of independent trait evolution and estimated the four-transition rate parameters $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, wherein double transitions from state 0,0 to 1,1 or from 0,1 to 1,0 are set to zero. We then ran a model of dependent trait evolution with eight parameters (q12, q13, q21, q24, q31, q34, q42, q43). To compare these non-nested models, we calculated their Bayes factor scores.

Data availability. All data generated in this article is available in the online Supporting Information.

Acknowledgements

We thank Jeremy Aroles for help with fieldwork, Benoît Chomicki for help with fieldwork and video, drone and photographic support, Alivereti Naikatini and Marika Tuiwawa for fieldwork assistance and permissions, Michael Maus for conducting the isotope measurements, Pepijn Kooij for discussion and Stuart West, Bill Wcislo and an anonymous reviewer for comments that helped improve the manuscript. This work was supported by grants from the Ammodo Foundation to E.T.K., as well as from the German Research Foundation (DFG) grants RE 603/20-1 and -2. GC was supported by a Glasstone research fellowship and a Junior Research Fellowship at the Queen's college, both at the University of Oxford, UK as well as a National Geographic grant (WW-133R-17) and currently by a NERC independent research fellowship (NE/S014470/1).

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Figure captions

Figure 1. Crop productivity varies across a light gradient and correlates with anti-herbivore defense efficiency. (A) Normalized number of food bodies per shoot along a light gradient. Inset shows food rewards (post-anthetic nectaries), with a *Philidris nagasau* worker foraging on one such food reward. (B) Herbivore damage on crops along a light gradient. (C) Ant patrolling activity on the domatium vs. shoot across the light gradient. Patrolling activity was measured as the percentage of ants present on the shoot (leaves, stem, food rewards [where most ants are found; c.f. ref. 16]) versus the domatium surface. (D) Linear relationship between the number of food bodies ($y = -0.012x + 1.182$; $R^2 = 0.40$, $P < 0.001$).

Figure 2. Ant nitrogen fertilization of crops along a light gradient. The boxplot shows the percentage of nitrogen in warty domatium walls where the ants defecate (see ref. 14) along a light gradient.

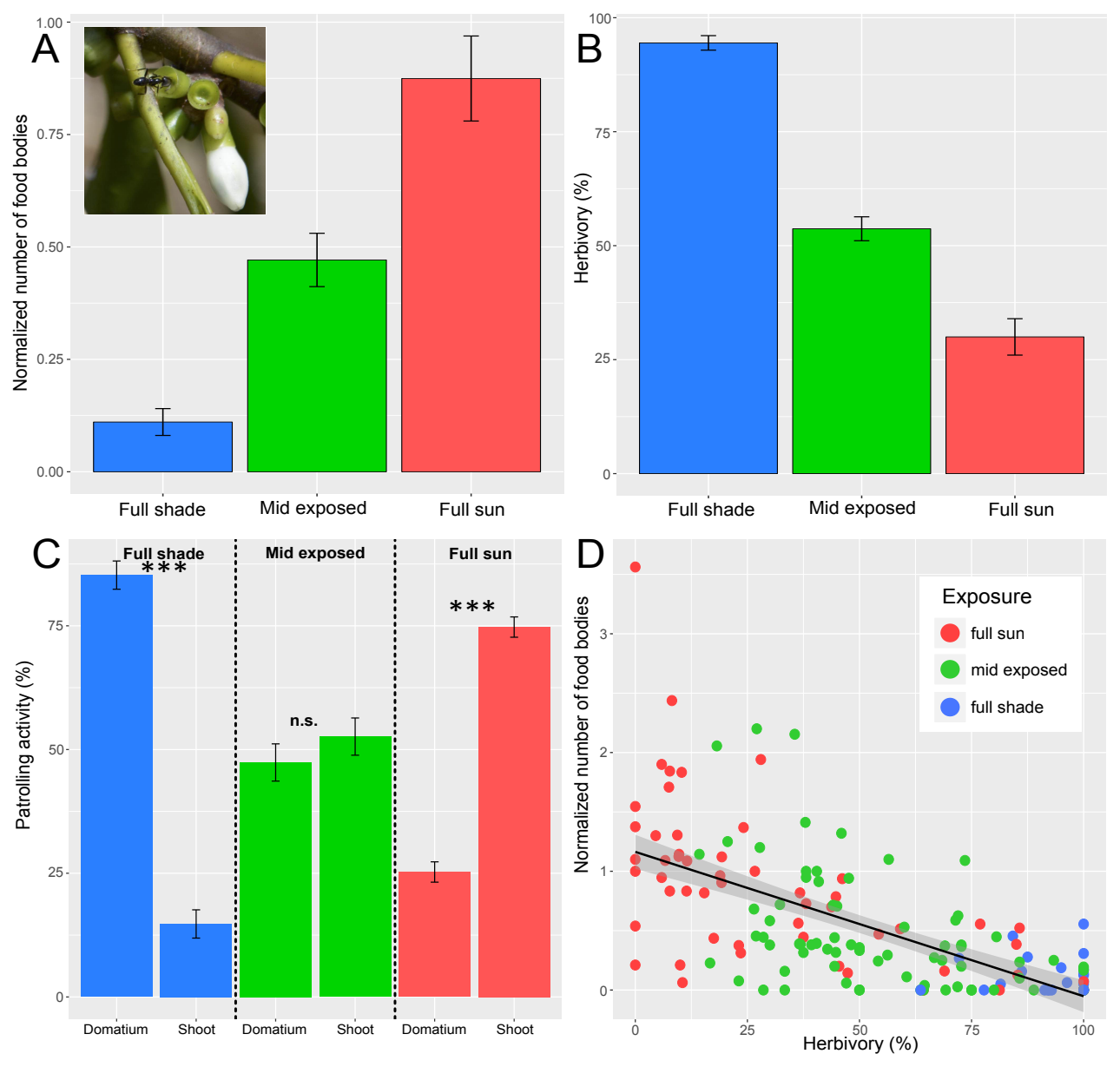
Figure 3. Models linking nutrition and defense via plant crop productivity (food rewards) to the light environment in *Squamellaria wilsonii* reveals tradeoffs. (A-B) Multinomial logistic models showing the probability of herbivory (A) and nitrogen content in warts (B) as a function of the light level.

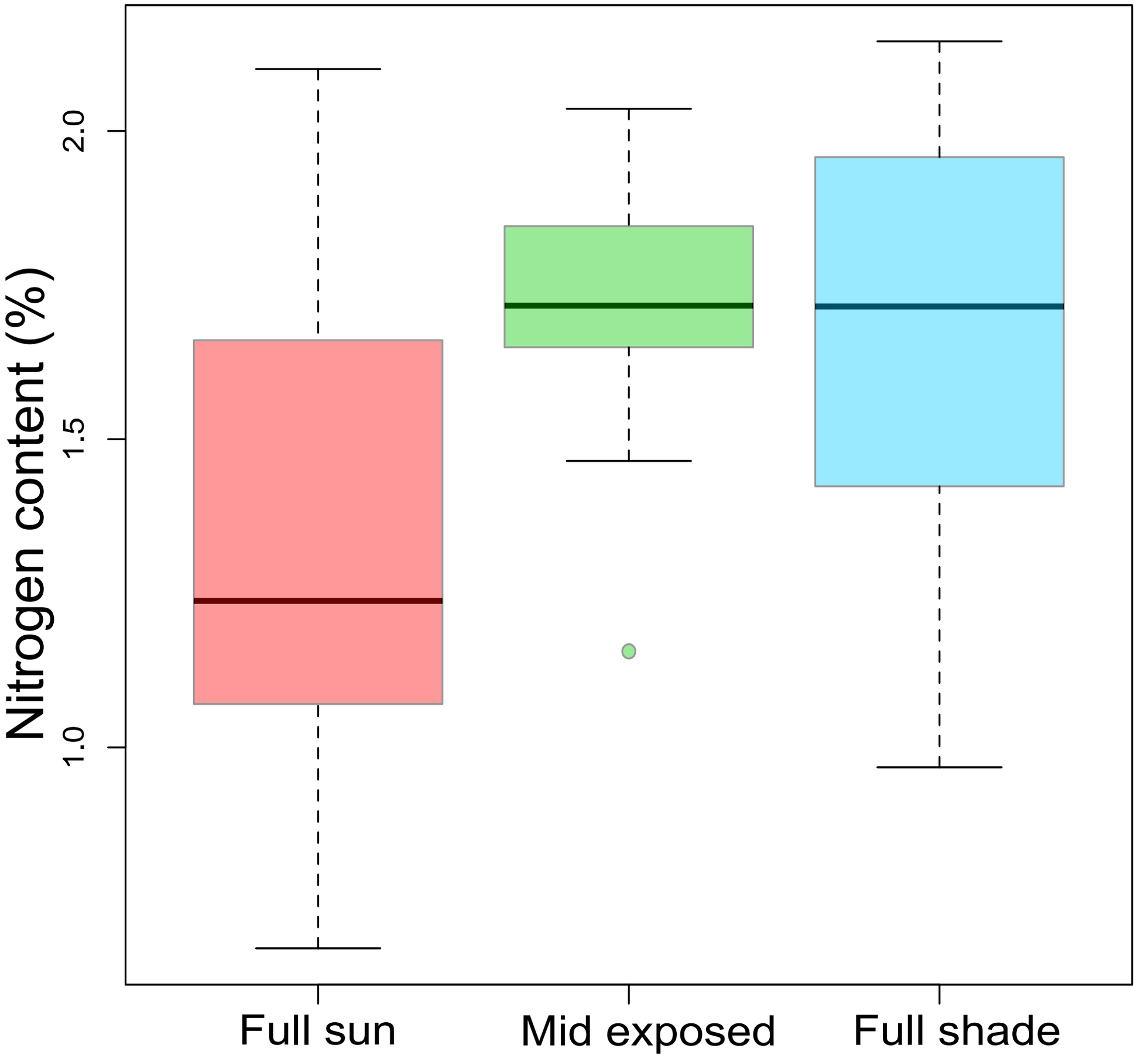
Figure 4. Farming ants control plant light niche over ecological and evolutionary time. (A) Light niches of 657 *Squamellaria wilsonii* plants occupied by 100 *Philidris nagasau* colonies in Taveuni. (B) Current (ecological) light niche of farmed vs. non-farmed Fijian *Squamellaria*. (C) Ancestral state reconstruction of light niche over a phylogeny of the Hydnophytinae (30), estimated from a maximum likelihood approach (35) with 1,000 simulations of character states, and a reverse-jump MCMC approach using 1,000 trees from the dating analysis (36), taking into account phylogenetic uncertainty (shown as probabilities above branches).

Online Supplementary Materials

Figs. S1-S12

784 **Tables S1-S2**


A
Normalized number of food bodies
1.00
0.75
0.50
0.25
0.00
Full shade
Mid exposed
Full sun
B
Herbivory (%)
100
75
50
25
0
Full shade
Mid exposed
Full sun
C
Patrolling activity (%)
75
50
25
0
Full shade
***
Mid exposed
n.s.
Full sun
***
Domatium
Shoot
Domatium
Shoot
Domatium
Shoot
D
Normalized number of food bodies
3
2
1
0
Exposure
full sun
mid exposed
full shade
0
25
50
75
100
Herbivory (%)


Nitrogen content (%)
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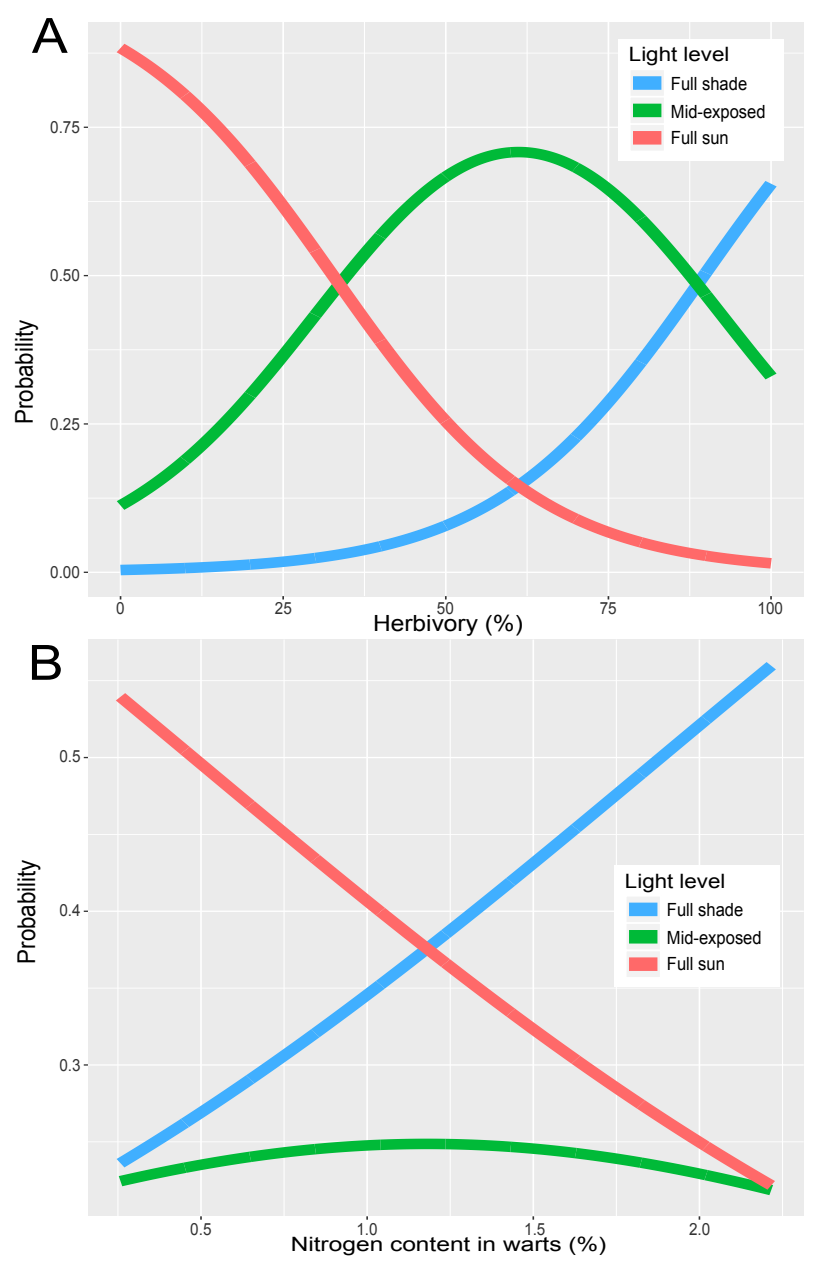
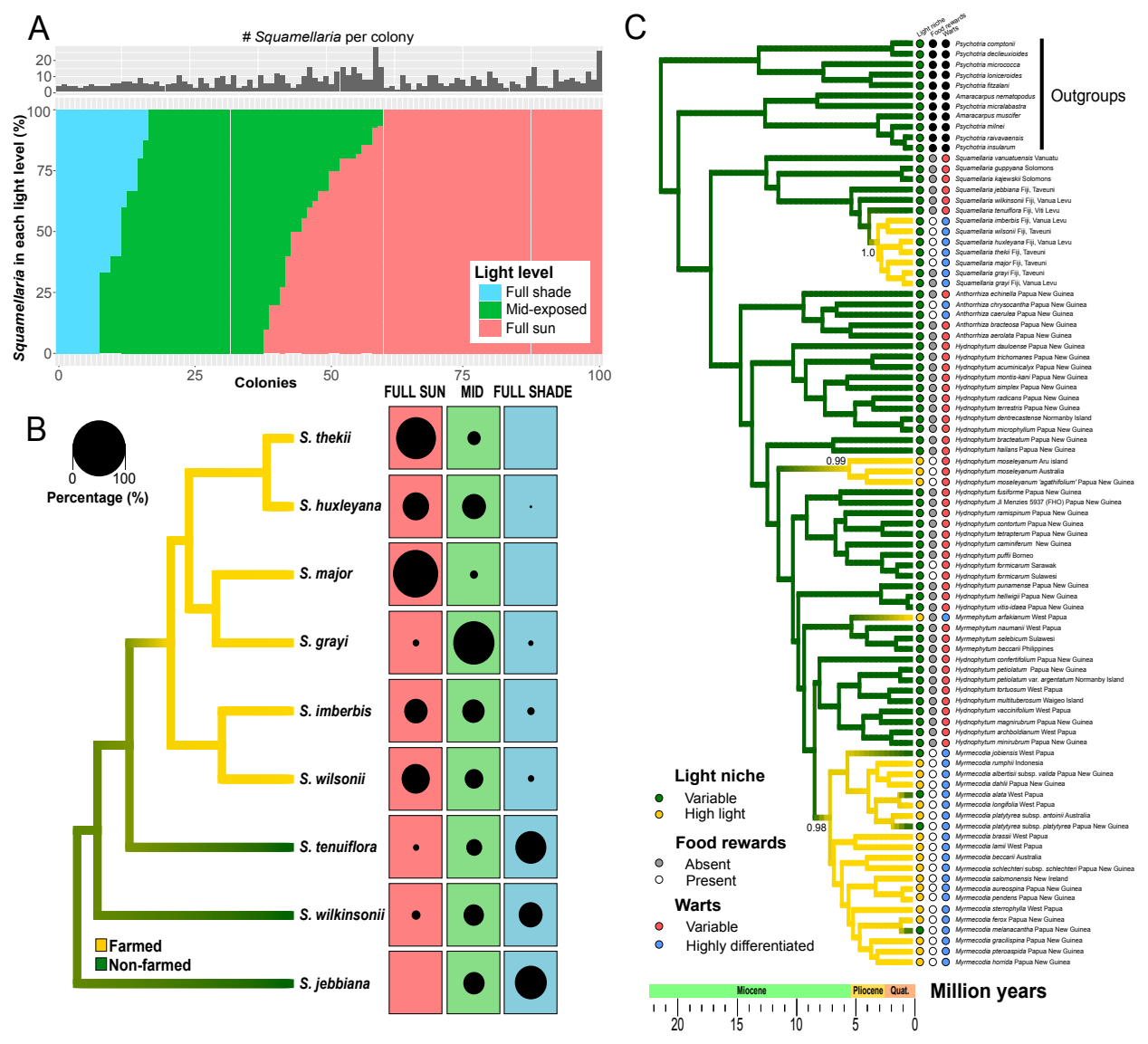
1.5
2.0
Full sun
Mid exposed
Full shade


A
Light level
Full shade
Mid-exposed
Full sun
Probability
0.75
0.50
0.25
0.00
0
25
50
75
100
Herbivory (%)
B
Light level
Full shade
Mid-exposed
Full sun
Probability
0.5
0.4
0.3
0.5
1.0
1.5
2.0
Nitrogen content in warts (%)


A
Squamellaria per colony
20
10
0
100
75
50
25
0
Squamellaria in each light level (%)
0
25
50
75
100
Colonies
Light level
Full shade
Mid-exposed
Full sun
B
0
100
Percentage (%)
FULL SUN
MID
FULL SHADE
S. thekii
S. huxleyana
S. major
S. grayi
S. imberbis
S. wilsonii
S. tenuiflora
S. wilkinsonii
S. jebbiana
Farmed
Non-farmed
C
Light niche
Food rewards
Warts
Outgroups
Psychotria comptonii
Psychotria declieuxioides
Psychotria micrococca
Psychotria ioniceroides
Psychotria fitzalanii
Amaracarpus nematopodus
Psychotria micralabastra
Amaracarpus muscifer
Psychotria milnei
Psychotria ravuvuensis
Psychotria insularum
Squamellaria vanuatuensis Vanuatu
Squamellaria guppyana Solomons
Squamellaria kajewskii Solomons
Squamellaria jebbiana Fiji, Taveuni
Squamellaria wilkinsonii Fiji, Vanua Levu
Squamellaria tenuiflora Fiji, Viti Levu
Squamellaria imberbis Fiji, Vanua Levu
Squamellaria wilsonii Fiji, Taveuni
Squamellaria huxleyana Fiji, Vanua Levu
Squamellaria thekii Fiji, Taveuni
Squamellaria major Fiji, Taveuni
Squamellaria grayi Fiji, Taveuni
Squamellaria grayi Fiji, Vanua Levu
1.0
Anthorrhiza echinella Papua New Guinea
Anthorrhiza chrysocantha Papua New Guinea
Anthorrhiza caerulea Papua New Guinea
Anthorrhiza bracteosa Papua New Guinea
Anthorrhiza aerolata Papua New Guinea
Hydnophytum dauloense Papua New Guinea
Hydnophytum trichomanes Papua New Guinea
Hydnophytum acuminicalyx Papua New Guinea
Hydnophytum montis-kani Papua New Guinea
Hydnophytum simplex Papua New Guinea
Hydnophytum radicans Papua New Guinea
Hydnophytum terrestris Papua New Guinea
Hydnophytum dentrecastense Normanby Island
Hydnophytum microphyllum Papua New Guinea
Hydnophytum bracteatum Papua New Guinea
Hydnophytum hailans Papua New Guinea
0.99
Hydnophytum moseleyanum Aru island
Hydnophytum moseleyanum Australia
Hydnophytum moseleyanum 'agathifolium' Papua New Guinea
Hydnophytum lusiforme Papua New Guinea
Hydnophytum JI Menzies 5937 (FHO) Papua New Guinea
Hydnophytum ramispinum Papua New Guinea
Hydnophytum contortum Papua New Guinea
Hydnophytum tetrapterum Papua New Guinea
Hydnophytum caminiferum New Guinea
Hydnophytum puffii Borneo
Hydnophytum formicarum Sarawak
Hydnophytum formicarum Sulawesi
Hydnophytum punamense Papua New Guinea
Hydnophytum heliwigii Papua New Guinea
Hydnophytum vitis-idaea Papua New Guinea
Myrmephytum arfakianum West Papua
Myrmephytum naumanii West Papua
Myrmephytum selebicum Sulawesi
Myrmephytum beccarii Philippines
Hydnophytum confertifolium Papua New Guinea
Hydnophytum petiolatum Papua New Guinea
Hydnophytum petiolatum var. argentatum Normanby Island
Hydnophytum tortuosum West Papua
Hydnophytum multituberosum Waigeo Island
Hydnophytum vaccinifolium West Papua
Hydnophytum magniruburn Papua New Guinea
Hydnophytum archboldianum West Papua
Hydnophytum miniruburn Papua New Guinea
Myrmecodia jobiensis West Papua
Myrmecodia rumphii Indonesia
Myrmecodia albertisii subsp. valida Papua New Guinea
Myrmecodia dahlii Papua New Guinea
Myrmecodia alata West Papua
Myrmecodia longifolia West Papua
Myrmecodia platytyrea subsp. antoinii Australia
0.98
Myrmecodia platytyrea subsp. platytyrea Papua New Guinea
Myrmecodia brassii West Papua
Myrmecodia lamii West Papua
Myrmecodia beccarii Australia
Myrmecodia schlechteri subsp. schlechteri Papua New Guinea
Myrmecodia salomonensis New Ireland
Myrmecodia aureospina Papua New Guinea
Myrmecodia pendens Papua New Guinea
Myrmecodia sterophylla West Papua
Myrmecodia ferox Papua New Guinea
Myrmecodia melanacantha Papua New Guinea
Myrmecodia gracilispina Papua New Guinea
Myrmecodia pteraspida Papua New Guinea
Myrmecodia horrida Papua New Guinea
Light niche
Variable
High light
Food rewards
Absent
Present
Warts
Variable
Highly differentiated
Miocene
Pliocene
Quat.
20
15
10
5
0
Million years