Research Ideas and Outcomes 8: e94784
doi: 10.3897/rio.8.e94784

Grant Proposal

Ant-plant symbioses trade-offs and its role in forest restoration projects

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Reviewable v 1

Received: 13 Sep 2022 | Published: 27 Sep 2022

Citation: Yek SH (2022) Ant-plant symbioses trade-offs and its role in forest restoration projects. Research Ideas and Outcomes 8: e94784. https://doi.org/10.3897/rio.8.e94784

Abstract

Ant-plant symbioses are complex between-species interactions found only in the tropical environment. Typically, in such symbioses, plants provide housing structures and food to their ant symbionts. In return, the ants protect their plants' host against herbivore attack and additional nutrients to help with plants' growth. These win-win interactions range from facultative to obligate mutualism. This proposal aims to test the three main mechanisms: (1) by-product benefits, (2) partner fidelity feedback and (3) partner choice in stabilising the ant-plant mutualism. Understanding the mechanisms are crucial as they form the foundation of the ant-plant distribution and growth, in other words - the health of the myrmecophyte (ants-loving) trees in the forest ecosystem. Hence, ant-plant symbioses are an ideal model system for investigating the effects of anthropogenic changes, such as deforestation and climate change on the outcome of ant-plant mutualistic interactions. This project attempts to identify the mechanisms regulating the mutualistic interactions and, in particular, identify the context in which such mutualistic interactions evolved and adapt to the changing environment. We hypothesise that there will be a higher diversity of obligate mutualistic ant-plant interactions in the undisturbed environment compared to degraded habitat. Furthermore, we expect there are different complexity of symbioses, involving multiple partners (ants-hemipteran insects-bacteria-fungi-plants) that deepen our understanding of how such symbioses can be stabilised. Finally, the deforestation combined with climate change in Southeast Asia will have a detrimental effect on ant-plant

symbioses, causing breakdown of mutualistic partners and invasion of cheater ant species that do not confer a protective advantage to their plants' host.

Keywords

Macaranga ant-plant, Malaysia, forest restoration, biodiversity conservation, species interactions, mutualism

List of participants

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Problem Statement

In Southeast Asia, human population growth leads to a dramatic increase in the exploited land surface, resulting in deforestation and various degrees of habitat fragmentation (Rudel et al. 2019). Moreover, climate change has been known to shift species distribution by ecological niche shifting, especially along environmental gradients (VanDerWal et al. 2012). The interaction stability of ant-plant mutualism has been under-studied from the perspective of land-use changes and climate change. This is mainly because the majority of ant-plant symbioses are under-explored, except for a few well-known examples from Africa and Central America tropics. We have no idea on the extent of diversity and interaction specificity of obscure ant-plant symbioses, especially in the Southeast Asian forests.

Hypothesis

The first hypothesis involves identifying the mechanisms regulating the degree of association. From literature, plants that actively recruit ants' symbiont are known to adjust the amount and composition of resources for ants. For example, when plants experience a higher rate of herbivore's damage, a higher quality of extrafloral nectary (EFN) is secreted, the concentrations of sugars and amino acids increased and sometimes even the overall number of food bodies is increased (Lange et al. 2017). These resources adjustment correlates with increased ants visitation. However, it was unclear whether the increased visitation rate of ant symbiont translates to improved protection. Phase one of the project will thus address the different mechanisms employed by plant and ant partners and whether the changes in mechanisms tip the balance of ant-plant symbiosis from mutualistic to parasitic interactions. We will test the hypothesis that change in food reward provided by host plant changes the ant-plant symbiosis from mutualistic to parasitic.

The second phase of the project addresses the question of how changes in habitat structure change the interactions' context. We hypothesise that ant-plant symbioses are important to maintain the health of their hosts' trees and hence have a beneficial effect on

trees distribution and growth. As a consequence to the land-use change and rapid deforestation of SE Asia forest, there will be a detrimental effect on the ant-plant symbioses through adaptive changes, such as the breakdown of partner's fidelity between ants and plants. We will test the hypothesis that proximity to the urban environment will bring in more opportunistic ant species (such as invasive ants) to steal the resources produced by the plants, displacing the native ant symbiont without offering the protection and nutritional services.

Research Questions

This project address two research questions:

1. What are the mechanisms regulating the mutualistic interactions in ant-plant symbioses and

2. In what context ant-plant mutualistic interactions evolved and adapt to changing environment, especially on forest fragmentation and climate change?

Literature Reviews

Ant-plant symbioses have been a textbook example of cooperation between individuals of different species (i.e. mutualism) (Del-Claro et al. 2018). From the trees' perspective, the benefit of having the ants' partners protect against herbivores, pathogens, encroaching vegetation and sometimes even essential nutrients for promoting trees growth. From the ants' perspective, they gain nesting spaces called domatia (i.e. hollow thorns, petioles, stems, rhizomes, tubers or modified leaves) and food such as extrafloral nectaries (EFN) and food bodies (i.e. nectaries and lipid-rich structures produced outside the flower solely to attract ants) from their trees' host (Blüthgen et al. 2007). The ants and the trees have co-diversification since the diversification and dominance of angiosperms 135-65 mya (Legendre and Condamine 2018). In the present day, many of ant-plant mutualisms are ant species-tree host specific and are only found in the tropics, leading to delicate between-species cooperative networks that together form the identity and diversity of the tropical forests' ecosystems. In Malaysia, there are 20+ species of *Macaranga* trees and *Crematogaster* ants interactions with most *Macaranga* found at the edges of forests and tree falls in the interior forests (Fiala et al. 1994). Some *Macaranga* species were utilised as restoration trees for habitat restoration projects (Rodrigues and Rodrigues 2014). The choice of *Macaranga* trees were deemed especially important in successful restoration. Yet, the survival and growth rate of *Macaranga* species depend heavily on its ants' partner (Blüthgen et al. 2007) and this is where this project will contribute to the understanding on how *Macaranga* ant plants trade-off in fragmented habitat.

Relevance to Government Policy

This research is in-line with both the Malaysia National Policy on Biological Diversity (2016-2025) and one of the 12 national Key Economic Areas. Malaysia National Policy on Biological Diversity (2016-2025) provides the direction and framework for us to conserve our biodiversity and use it sustainably in the face of the increasingly complex challenges. By understanding the biodiversity and the interaction stability of ants-plants, the government could better manage the health of rainforest that is part of the ecotourism industry, one of the 12 National Key Economic Areas.

Objectives of the Research

This project has two objectives.

First objective: we will identify and select several species of *Macaranga* ant-plant species to examine the mechanisms regulating the mutualistic interactions in ant-plant symbioses. We will perform trade-off experiments and biochemical analysis assays on the plant-derived resources to test the response from their ants' symbiont.

Second objective: To examine under what context ant-plant mutualistic interactions evolved and adapt to the changing environment, especially on forest fragmentation and climate change. We will simulate the effect of land-use change (such as forest fragmentation) and trees planting as restoration to examine the colonisation of their ants' symbiont.

Methodology

Phase 1: Mechanisms regulating the degree of ant-plant associations

In the first phase, we will examine how plants regulate their resources to attract beneficial ants and to deter opportunistic ants. Some ant-plants are known to adjust the sugar and amino acid content when they experience higher herbivory damage (Lange et al. 2017). We will examine whether this adjustment applies to all ant-plant mutualism or only obligate ant-plant mutualism. We expected that the changes in the resources provided have the potential to tip the balance of ant-plant symbioses from mutualistic to antagonistic interactions. This phase involves close collaboration with a plant biochemist to chemically characterise the composition of plant-derived resources (i.e. EFN and food bodies) and resources manipulation experiments to examine the plasticity of ant-plant associations.

Phase 2: Adaptation of ant-plant symbioses to changing landscape and climate change

The final phase of the project examined the adaptation and evolution of ant-plant symbioses of a SE Asia forest. In Central America, habitat fragmentation had little effect on the density and diversity of ants and plants (Guo et al. 2019), but different ant-plant interactions showed varying resilience to habitat changes (Gibert et al. 2019). Furthermore, the impact of climate change on ant-plant symbioses has yet to be investigated. We

hypothesise that obligate ant-plant partnership would confer the most benefit to maintain the health of their hosts' trees and also will be the most susceptible to changing landscape and climate change. As a consequence, we expect to see adaptation in ant-plant symbioses (e.g. change of ant partners, reduced resource productions, invasion of cheater symbiont etc.). This phase involves a large-scale survey to discern the vulnerability of obligate ant-plant symbioses.

Novel theories/New findings/Knowledge

A wide range of ant-plant symbioses diversity exists across different habitats: we hypothesise that there is a gradient of associations, ranging from opportunistic, facultative, mutualistic to even parasitic ant-plant associations with both partners trying to best the other one by gaining the most benefit out of the associations. We expect this diversity will manifest most in pristine forest habitat where there has been an extended period of undisturbed co-evolution. In a disturbed environment, ant-plant associations will most likely be opportunistic with many ant species colonising either one host plant or many host trees, without protection against herbivores and nutritional mutualism.

Ant-plant regulation mechanisms to optimise associations: we hypothesise that the more specialised the ant-plant symbiosis, the finer tuning the ants and its plant partners can regulate the resources contributing to the partnership. For example, *Macaranga* trees that have a waxy stem selectively choose the 'right' ant partner because only their obligate ant symbiont, *Crematogaster* spp. can climb the waxy stem (Quek et al. 2004). In contrast, opportunistic and facultative ant-plant symbiosis will most likely lack selection mechanisms in regulating resources provided to the partnership.

Changes in ant-plant symbioses in the changing land-use practice: we expect obligate ant-plant symbioses are sensitive to land-use changes. In an ants-exclusion experiment, we expect host plants will suffer high herbivore attack and/or pathogen load without their obligate ant partners. At the other end of ant-plant symbioses, host plants will have a better defence system without their opportunistic/facultative ant partners (Fiala et al. 1994). These findings will be useful as bioindicators of the health of the forests and it could provide actionable information when it comes to choosing ant-trees species for forest restoration projects.

Impact on Society, Economy and Nation

Since the formulation of the first National Policy on Biological Diversity in 1998, Malaysia has undergone a significant population increase. As the country's transition to a developed, high-income nation, our biodiversity suffers. Many species are now facing threats of extinction. Compounded with global issues like climate change, Malaysians generally have a lack of awareness on the importance of biodiversity throughout the country, as well as a significant knowledge gap. This project increase the knowledge between interactions networks that form the important tenet of biodiversity in Malaysia and also provide

actionable plan on how biodiversity can be effectively conserved through restoration projects.

Budget

Table 1 for each category budget allocation.

Table 1.

Budget allocated by the funding agency in Ringgit Malaysia for 'Ant-plant symbioses trade-offs and its role in forest restoration projects'.

Description	Allocated (RM)
Vote 11000 - Salary and Wages	43200
Vote 21000 - Travelling and Transportation	21000
Vote 24000 - Rental	6000
Vote 27000 - Research Materials and Supplies	8000
Vote 29000 - Professional Services	30000
Vote 35000 - Accessories and Equipment	5000
TOTAL	113200

Timeline

Refer to Fig. 1 for projected timeline. However, the project is now being extended to November 2022 due to the pandemic lockdown.

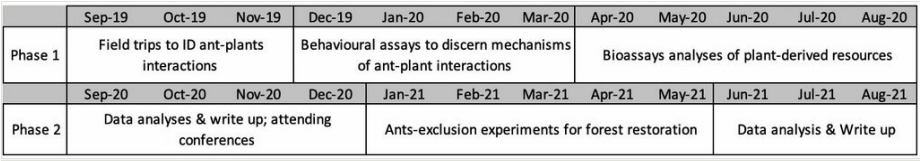

Figure 1. doi

Projected timeline for 'Ant-plant symbioses trade-offs and its role in forest restoration projects'

Funding program

Fundamental Research Grant Scheme (FRGS)

Grant title

Ant-plant symbioses trade-offs and its role in forest restoration projects.

Hosting institution

Monash University Malaysia

Author contributions

SHY was the main PI and administrator from 2019 to March 2022. Grant administration was taken over by ZHW (the participant) after SHY moved her hosting institution to Universiti Malaysia Sabah.

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