

DAVIS EXPEDITION FUND

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title:	Understanding the genetics of flexistyly and genetic diversity in the genus <i>Alpinia</i> Roxb. (Zingiberaceae)
Travel Dates:	28 th May 2018- 17 th July 2018
Location:	India (Assam and Arunachal Pradesh)
Group Members:	Surabhi Ranavat
Aims:	Collection of leaf tissues, rhizomes and herbarium specimens of <i>Alpinia</i> species for flexistyly and population genetic analysis.

Outcome (a minimum of 500 words):-

Understanding the genetics of flexistylly and genetic diversity in the genus *Alpinia* Roxb. (Zingiberaceae)



Surabhi Ranavat



THE UNIVERSITY
of EDINBURGH



Royal
Botanic Garden
Edinburgh



Background

Angiosperms possess many remarkable traits that enable them to reproduce successfully. Among many such characters are stylar polymorphisms that promote insect-mediated cross-pollination and prevent pollen wastage due to interference between male and female function within a bisexual flower (Barrett, 2010). One such polymorphism is called flexistyly, which was discovered in the genus *Alpinia* from the ginger family (Zingiberaceae). It is a unique floral dimorphism where the morphs can either be cataflexistylous (protandrous), in which the anthers disperse pollen in the morning, while the style is curved above the anther, or they can be anaflexistylous (protogynous), in which the style is curved below the anther while the anther does not disperse pollen. Around midday, the style of the cataflexistylous flower curves below the anther and receives cross pollen whereas the anther halts pollen release and the style of the anaflexistylous flower curves above the anther and the anthers begin to release pollen. (Li et al., 2001a, Barrett, 2002). Most of the flexistylous species so far have been reported from South-East Asia and China and a comprehensive study of their pollination and reproductive ecology has been done (Li et al., 2001b, 2002, Zhang et al., 2003, Takano et al., 2005, Sun et al., 2011) but little is known about the genetic basis of this trait.

The other interesting aspect of *Alpinia* species is that they have a disjunct distribution in India. They are found in the wet zones of India, i.e., the South-West and North-East. In this case, species cohesion may be maintained by contemporary gene flow across the two regions or this distribution could be historical, resulting due to the formation of dry zones in central India (Mani, 1974). An understanding of how these species are maintained in India is essential to understanding the evolution of species within this genus. Having collected *Alpinias* from one state in South India and 5 states in North-East India, this fieldwork was aimed at collecting specimens from 2 more states in the North-East.

Aims

The aims of this fieldtrip were to collect leaf tissues, herbarium specimens and rhizomes to address the following objectives:

- Elucidating the genetic basis of flexistyly.
- Understanding species cohesion of widespread *Alpinia* species showing a disjunct distribution.

Methods

Information about the location of the species of interest was collected from local herbaria such as Botanical Survey of India in Itanagar and Shillong. Based on this information, leaf tissues, rhizomes, and herbarium specimens were collected.

Results

242 samples of leaf tissues along with rhizome and herbarium specimens of *Alpinia* and other species of Zingiberaceae were collected in the states of Assam (6 districts), Arunachal Pradesh (3 districts) and Meghalaya (1 district). Species such

as *A. nigra*, *A. malaccensis*, *A. galanga* and *A. blepharocalyx* were collected (Figure 1). The collection permit for Assam was only for unprotected areas therefore most of the plants were collected from roadsides.

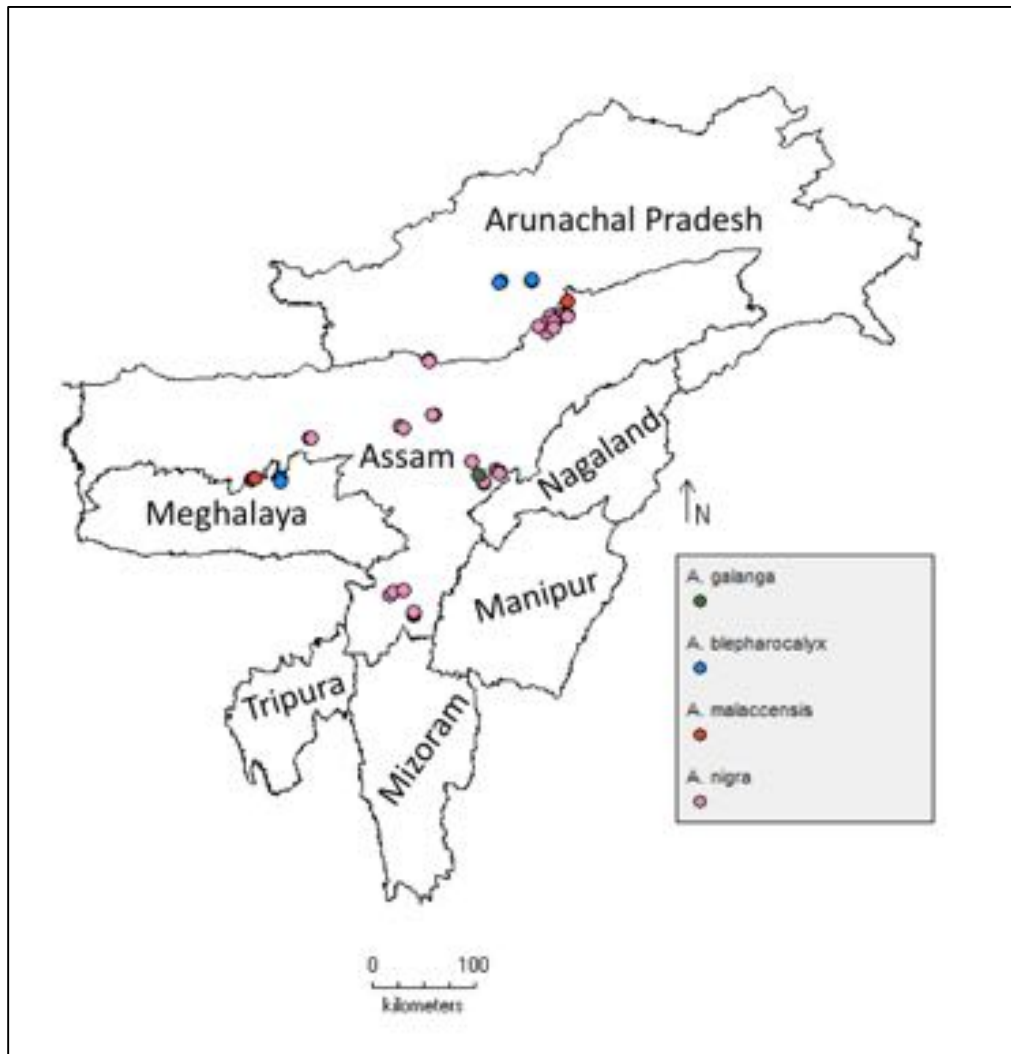


Figure 1: Collection localities in North-East India

53 anaflexistylous, 61 cataflexistylous and 5 unknown leaf tissues were collected from Pakke Tiger Reserve in East Kameng district, Arunachal Pradesh (Figure 2 and 3).

The herbarium specimens and rhizomes were deposited at Indian Institute of Science Education and Research, Bhopal (IISERB).

Species such as *Alpinia nigra* were found in marshy areas in the lowlands of Assam, Arunachal Pradesh and Meghalaya. *A. malaccensis* and *A. blepharocalyx* were found on slopes near light gaps at an elevation of 300-1000m. These species were found to be fruiting. *A. galanga* was found only in cultivation.

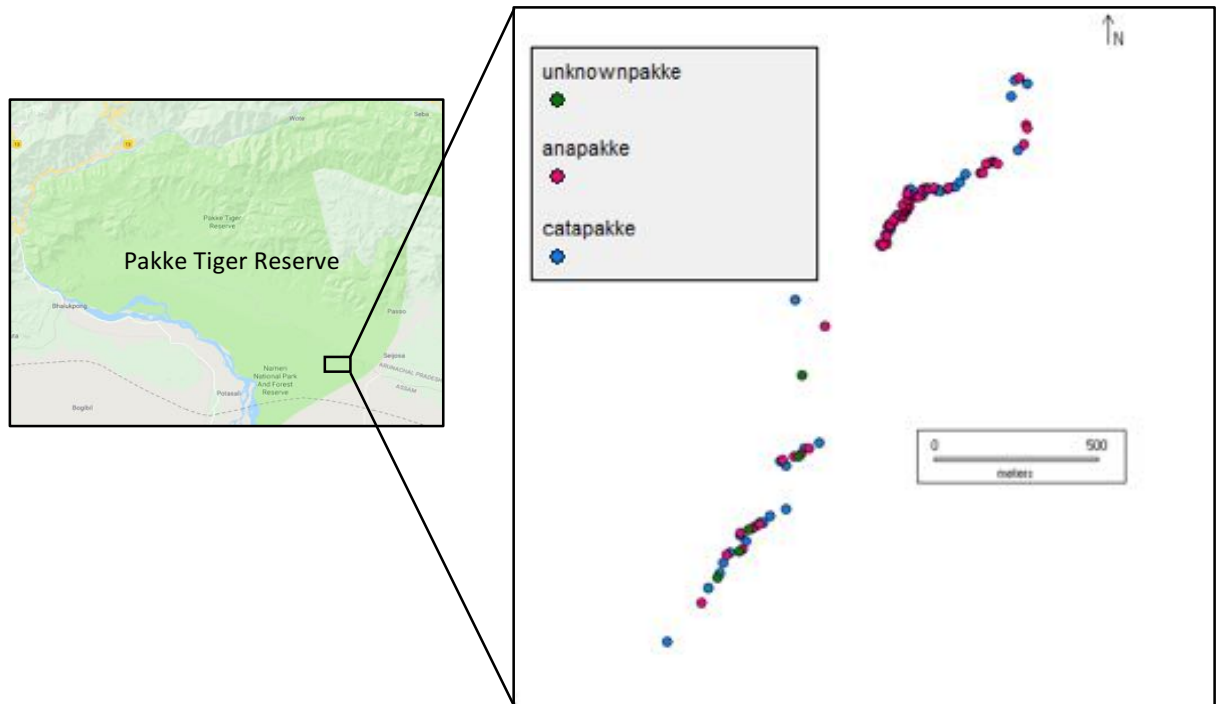


Figure 2: Map of the ana- and cataflexistylous morphs collected from Pakke Tiger Reserve, Arunachal Pradesh.



Figure 3: *Alpinia nigra* at Pakke Tiger Reserve, Arunachal Pradesh. a- Habitat, b- Cataflexistylous morph, c- Anaflexistylous morph.

Itinerary

Date	State	Description
28th May	-	Flight from Edinburgh to Mumbai
1st June	Madhya Pradesh	Flight from Mumbai to Bhopal and collection of fieldwork equipment from IISERB
2nd-3rd June	Assam	Train and flight to Guwahati via New Delhi
4th June	Assam	Visit to the PCCF Office, Panjabari
6th June	Assam	Fieldwork in Chandrapur, Kamrup (M) dt
7th June	Assam	Overnight Bus to Silchar
8th June	Assam	Visit to the Forest office in Silchar
9th June	Assam	Fieldwork in Silchar-Purabthol-Sonai-Silchar
10th June	Assam	Fieldwork in Silchar-Hailakandi-Katakhal-Silchar
11th June	Assam	Train to Lumding and Diphu from Silchar
12th June	Assam	Visit to the Forest office in West Karbi Anglong and central range office
13th June	Assam	Fieldwork in recreational park and Dhansiri road + visit to Manja
14th June	Assam	Fieldwork near Manja and Silveta falls
15th June	Assam	Fieldwork along Dimapur road from Manja, and Siloni
16th-17th June	Assam	Train to Guwahati+ Rest day
18th June	Assam	Taxi to Nagaon and visit to the Forest Office in Nagaon
19th June	Assam	Fieldwork in Salona and North Dejoo
20th-23rd June	Assam	Fieldwork in Barhampur
24th June	Assam	Taxi from Nagaon to Kamakhya station and train to North Lakhimpur
25th June	Assam	Visit to the Forest range office in North Lakhimpur
26th June	Assam	Visit to DFO office Japisajia
27th June	Assam	Fieldwork at Dullong and Kakoi Reserve Forests
28th June	Assam	Fieldwork in Pohmura, Kimin, Lilabari airport
29th June	Arunachal Pradesh	Taxi from North Lakhimpur to Itanagar and visit to BSI Itanagar
30th June-1st July	Arunachal Pradesh	Rest day
2nd July	Arunachal Pradesh	Visit to PCCF office, Itanagar

3rd July	Arunachal Pradesh	Fieldwork from Itanagar to Ziro
4th July	Arunachal Pradesh	Fieldwork from Ziro to Tamen
5th July	Arunachal Pradesh	Fieldwork from Ziro to Palin
6th-10th July	Arunachal Pradesh	Taxi to Itanagar and Seijosa
11th July	Assam	Travel to Guwahati via Tezpur
12th July	Assam	Data entry + specimen sorting
13th July	Assam	Fieldwork from Guwahati to Chandubi Lake
14th July	Assam/Meghalaya	Fieldwork from Guwahati to Nongkhlaw
15th July	Madhya Pradesh	Flight to Bhopal via Delhi
16th July	Madhya Pradesh	Sort tissues, rhizomes and herbarium specimens in IISERB
17th July	Maharashtra	Flight to Mumbai
18 th July- 2 nd August	-	Personal Holiday + Zingiberales Symposium
3 rd August	-	Return to Edinburgh

Awards Granted

Heredity Fieldwork Grant- £1,440

Davis Expedition Fund- £2,405

Expenses

Description	Expenses (INR)	Expenses (£)
Flights	86159.8	960.5328874
Trains	13920	155.1839465
Taxi	68361	762.1070234
Food	14292	159.3311037
Accommodation	42731	476.3768116
Consumables	4738	52.82051282
Permits and entry fees	2560	28.53957637
Total		2594.891862
Total Amount received		3,845
Remainder		1,250.108

INR 89.70= £1

The remainder will be returned to the Davis Expedition Fund.

Future Plans

- *De novo* genome assembly of *Alpinia nigra*.
- Bulk segregant analysis will be carried out after pooled sequencing of the ana- and cata-morphs.
- Genotyping by Sequencing (GBS) will be done for the population genetic analysis for material collected from this and the previous field trip.

Acknowledgements

I would like to thank Dr Vinita Gowda (Assistant Professor, IISERB) for her guidance during fieldwork. I would also like to thank Susnata Salony, Ajith Ashokan, Preeti, Saket Shrotri and Prasanna NS (BS-MS and PhD students, IISERB) for helping me in the field. I am grateful to all the local and regional forest department personnel for granting permissions and assisting me to carry out the necessary work. Lastly, I would like to thank Davis Expedition Fund and Heredity Fieldwork Grant for their generous support for my field trip.

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Appendix



Figure 4: *Alpinia nigra* found in Silchar district, Assam. a- Habit, b- Habitat, c- flower, d- inflorescence and flower being pollinated by a carpenter bee.



Figure 5: *Alpinia malaccensis* found in Dullong Reserve Forest, North Lakhimpur district, Assam. a- Habit, b- Habitat, c and d- Infructescence.



Figure 6: *Alpinia blepharocalyx* found in Ziro district, Arunachal Pradesh. a- Habit, b- Habitat, c and d- Inflorescence.



Figure 7: *Alpinia galanga* (cultivated) in Diphu Recreational Park, Karbi Anglong district, Assam. a- Habit, b- Habitat, c- Inflorescence, d- flower.