

**Pollen and Pollinator Limitation of Seed Initiation  
in *Etilingera littoralis* (J. König) Giseke (Zingiberaceae)  
in Klong Klai Basin, Khao Nan National Park, Thailand**

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**ABSTRACT**

Pollen and pollinator limitation of seed initiation in *Etilingera littoralis* (J. König) Giseke (Zingiberaceae) in Khlong Klai Basin, Khao Nan National Park, Nakhon Si Thammarat Province, was investigated using hand and open pollination in 3 stations (A, B and C). Stations A and B are located inside the National Park area, whereas station C is outside the National Park area. Stingless bees were the only pollinators observed at station C. The number of seeds initiated from the hand-pollinated flowers were compared to those of seeds initiated from open-pollinated flowers. The results showed that there was a pollen limitation in stations A ( $T = 2.802$ , *Sig 2-tailed* = 0.012) and B ( $T = 2.524$ , *Sig 2-tailed* = 0.021) on mean seeds/ovules (S/O) ratios. In station A, pollen limitation was slightly greater (mean difference = 0.547) than station B (mean difference = 0.501). In station C, pollen limitation was not significant ( $T = 0.410$ , *Sig 2-tailed* = 0.550) because there was small stingless bees, *Trigona* sp. that pollinated the flowers. However, a resource limitation in station C was found on mean ovules per flower (mean difference A,B = 9.893, mean difference A,C = 31.734 and mean difference B,C = 21.841).

**Keywords:** Pollinator, pollen limitation, *Etilingera littoralis*-  
(König) Giseke, Zingiberaceae, *Trigona*

## INTRODUCTION

Pollination syndrome is a term for the descriptive interrelation between flower and pollinator. There are five aspects of flowers needed for any given type of syndrome. They are floral color, scent, time of flowering, structure and rewards. On the pollinator side, three main aspects are involved i.e. sensory capacity, behavior and diet. These factors are interrelated in the success of any pollination [1]. Rewards for the pollinator from the flower are mainly two kinds, nectar or pollen [1].

Reports of pollinators in the family Zingiberaceae are scant. *Hedychium coronarium* is pollinated by hawkmoths while *H. coccineum* is pollinated by butterflies [2]. Large bees (*Euglossine*, *Centris* and *Bombus*) are found to be the pollinators of the flower of *Alpinia zerumbet* and *Xylocopa* species as the pollinators of *A. malaccensis* and *A. hookeriana*. *Etilingera elatior* is found to be visited by birds of Nectariniidae and butterflies. *Renanthera* spp. are pollinated by hummingbirds [3]. *Etilingera brevilabris* and *Hornstedtia tomentosa* have *Arachnothera* spp. (Nectariniidae) as the pollinator. The latter species of Zingiberaceae both have red flowers and basal inflorescences. Small traplining bees (*Nomia* and *Trinchostoma* of Halictidae) are also observed on *Amomum polycarpum* and three species of *Boesenbergia*. Medium size traplining bees (*Amegilla*) are the pollinators of *Amomum gyrolophos*, *Plagiostachys crocydocalyx* and *Globba brachyanthera* [4]. A recent study by Sakai *et al.* [5] identified three pollination groups, namely spiderhunters (two species), *Amegilla* bees (two species) and halictid bees (four species) as the pollinators of 29 species of Zingiberaceae in Borneo. Three species of *Etilingera* and five other species of Alpinieae, were pollinated by spiderhunters, while 12 species (two *Costus* spp., *Globba brachyanthera*, *Zingiber longipedunculatum* and the rest of Alpinieae) were pollinated by *Amegilla* bees. Halictid bees were found to be pollinators of three species of *Boesenbergia* and seven species of Alpinieae [5].

Many plants regularly produce more flowers than fruits [6-8] and far more pollen than ovaries [9]. These excess flowers could serve to increase male reproductive success by attracting vectors to disseminate pollen. The pollinator limitation hypothesis suggests that inadequate pollinator service causes a reduction in fruit set [10,11]. Pollen limitation occurs when plants produce fewer fruits and/or seeds than they would with adequate pollen receipt [8]. Pollen limitation is demonstrated empirically when supplemental pollination (hand-pollinated) of flowers increases their female fertility compared to open-pollinated controls. Many species of flowering plants rely on mutualistic interactions with animal pollinators for sexual reproduction [9]. If pollinators fail to deliver an adequate quantity and quality of pollen, then

plants will mature fewer seeds. The magnitude of pollen limitation, a reduction in fruit or seed set as a result of inadequate pollen deposition, is often highly variable among populations of single species [10-12]. When flowers receive insufficient pollen and not all ovules are fertilized, the ratio of seeds to ovules is less than 1. In addition to pollen limitation, several other factors can influence variation in seeds/ovules (S/O) ratios including resource limitation and genetic load [13]. The resources limitation hypothesis suggests that female reproductive success may be limited by inadequate resources (stored food reserves, minerals, light and water) that prevent maturation of more fruits [6-8].

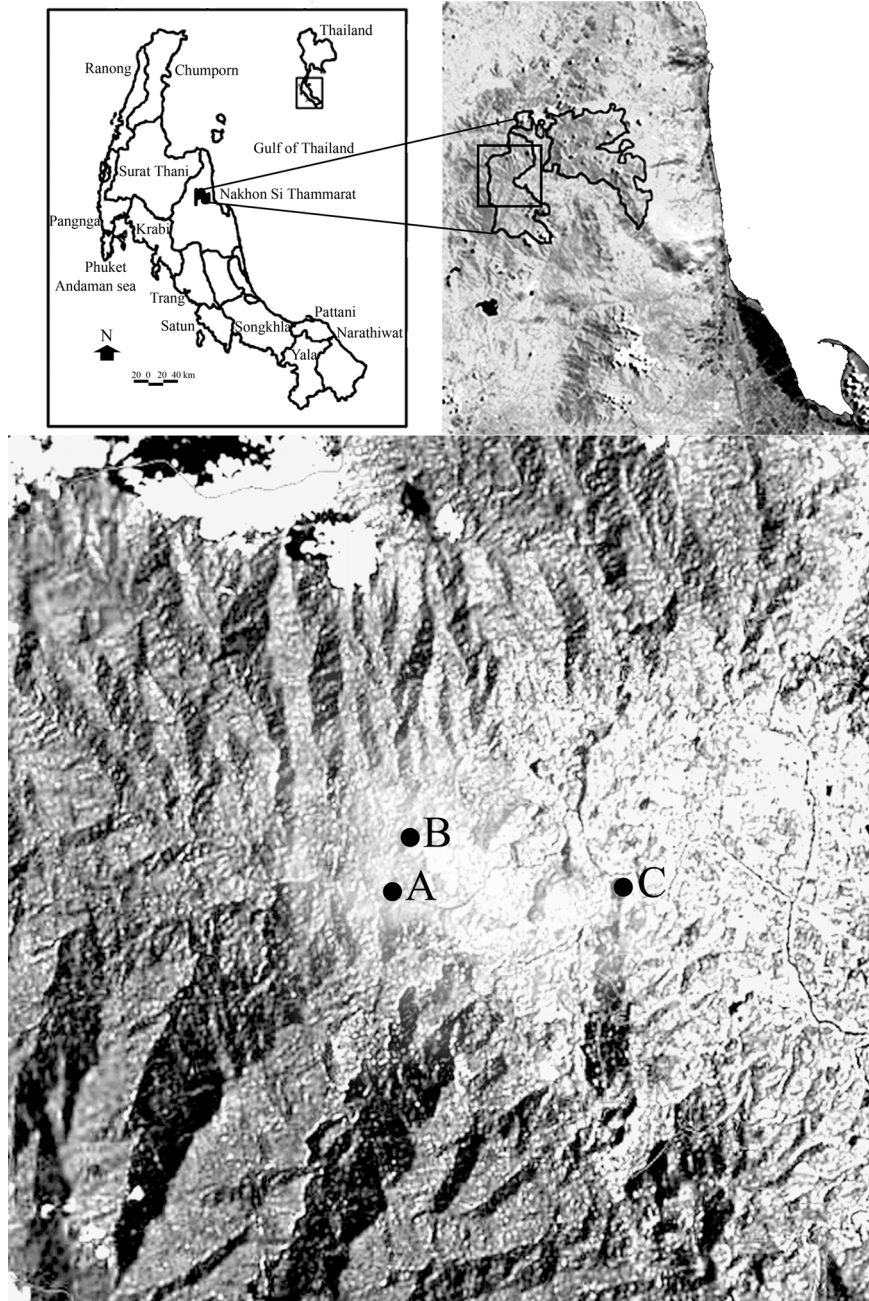
The purpose of this study was to determine whether pollination or resources limit seed initiation in populations of *Etilingera littoralis* (König) Giseke growing in highly disturbed habitats compared to intermediately disturbed habitats. In addition, we aimed to determine whether pollinator and pollination syndrome of *E. littoralis* was present or not.

## MATERIALS AND METHODS

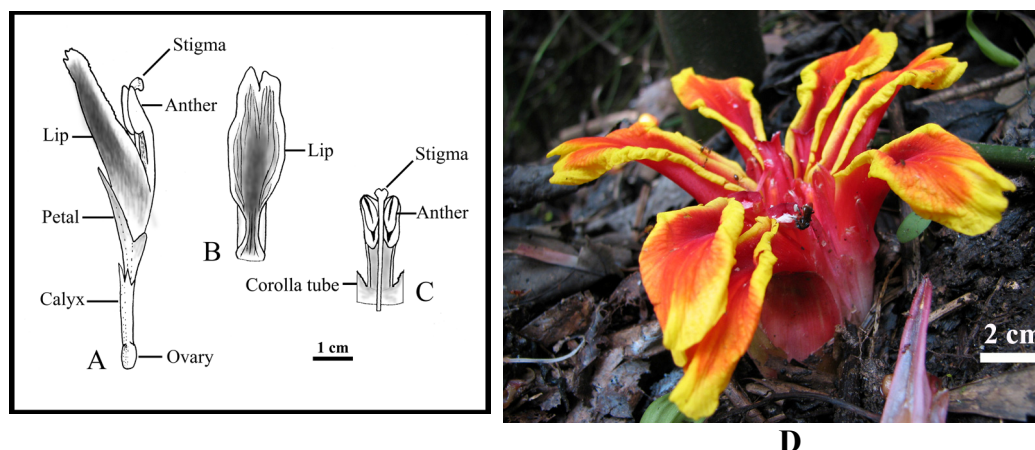
**Study site**-The flower pollination was conducted at Klong Klai Basin, Khao Nan National Park, Nakhon Si Thammarat Province, Thailand. Three stations, namely station A, B and C, were chosen mainly on their availability, to compare the effect of human disturbance on the pollen- and pollinator-limitation of *E. littoralis* (**Figure 1**). Stations A and B, are located inside the National Park (Station A: 8° 48' 16" N 99° 34' 07" E, 220 m in altitude and Station B: 8° 48' 18" N 99° 34' 02" E, 220 m in altitude). These areas are a tropical moist evergreen forest and an intermediate disturbed area near rubber plantation and rambutan orchard, respectively. Station C is located outside the National Park (8° 48' 07" N, 99° 34' 26" E, 220 m in altitude). The area is a disturbed area, located next to the road, and is approximately 2 kilometers apart from stations A and B. The habitat of *E. littoralis* at all three stations is open areas, close to streams (~ 2 - 5 m).

**Floral morphology**-The inflorescences of *E. littoralis* are short and compact (**Figure 2**). They emerge from a subterranean rhizome to produce open flowers at the soil surface. Flowers appear bright red and yellow due to the pigmentation of the expanded petaloid staminode. Flowers produce a single fertile stamen; the three other stamens have fused into a lip, a broad petaloid staminode characterized by a prominent landing platform. The staminode and stamen are fused at the base into a tube. The style is slender and is surrounded and supported by the anthers along its 4.2 - 5 cm length.

The stigma is exerted approximately 5 - 6 mm beyond the tip of the anther (**Figure 2**). The flowers are protogynous in which stigma receptivity precedes anther dehiscence for 2 - 3 h.



**Figure 1** Maps of Khao Nan National Park, and all three stations. (Adapted from Google Earth 2006)



**Figure 2** Floral structure of *Etilingera littoralis* (König) Giseke. (a) flower with bract and bracteole removed; (b) lip; (c) anther and stigma and (d) flower within inflorescence.



**Figure 3** Stingless bee, *Trigona* sp., collecting pollen and nectar at station C.

**Experimental design and methods**-To investigate pollen and pollinator limitation of seed initiation in *E. littoralis*, we conducted the experiments using 20 inflorescences at each station. The 20 inflorescences were equally divided 10:10 for treatments with either: (1) open-pollination, in which all flowers on an inflorescence were un-manipulated or (2) hand-pollination, in which all flowers on an inflorescence were cross-pollinated by hand. Pollen grains were collected from a plant, located > 5 m away, directly from the anther using a needle and forceps. The experiment was conducted in

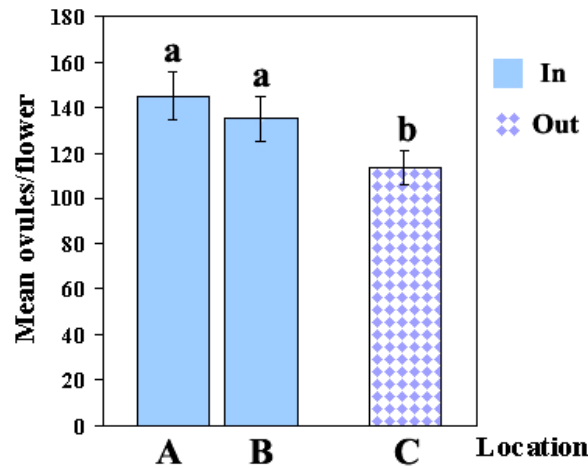
May 2006 during the flowering season. We performed all pollinations in the morning (08:00 - 11:00 am), when most natural pollination occurs. The pollinator visitations were also recorded for all inflorescences by naked eyes. A week after pollination, the number of ovules and seed initiation were counted from three randomly marked flowers in each inflorescence.

**Statistical analyses**-Statistical analyses were carried out using SPSS 11.5 for Windows. We conducted T-Test on the number of ovules number per flower and the total number of flowers (ovaries) in inflorescence from each location to detect differences between the two treatment groups. We used One-way Analysis of Variance (ANOVA) to detect the effects of location and pollination treatment (hand- vs. open-pollinated) on the mean ovule number per flower. In addition, we used Post Hoc Test; Dunnett T3, to detect differences in natural resource and/or genetic load between plants growing in the different areas (A, B and C). We conducted T Tests on inflorescences within each location to detect differences between pollination treatments with respect to the mean proportion number of seeds initiated (S/O ratios) per fruit in the 3 different studied locations.

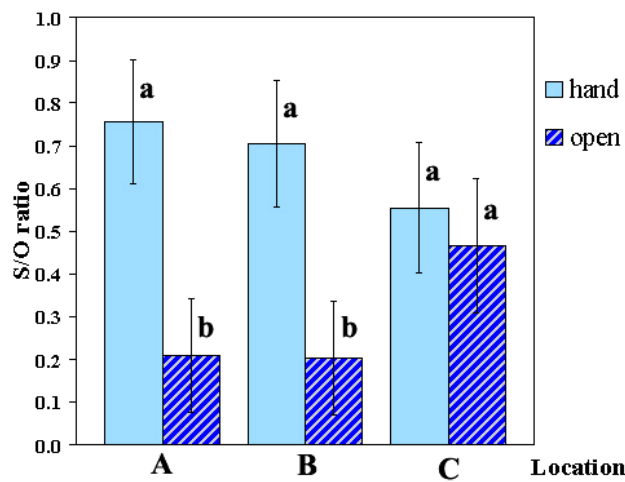
## RESULTS

**Pollinator limitation**- Birds or spiderhunters were not observed at all 3 stations. However, stingless bees, *Trigona* sp., were the only pollinators observed at station C. Stingless bees were observed to collect pollen and nectar from every flower in the inflorescence, spending about 20 - 30 seconds per flower (**Figure 3**). The average body size measured from ten samples is 6 - 7 mm in length and 1 - 2 mm in width.

**Pollen and resource limitation**- The Kolmogorov-Smirnov test indicates that the ovule numbers per flower are distributed normally ( $Sig = 0.200$ ). Within each station, plants chosen for the two treatments were similar in the ovule numbers per flower and the total number of flowers in inflorescences ( $Sig_A = 0.416$ ,  $Sig_B = 0.095$  and  $Sig_C = 0.054$ ). One-way ANOVA tests show significant differences in the mean number of ovules per flower among stations ( $F = 4.192$ ,  $Sig\ 2-tailed = 0.009$ ). Dunnett T3 show significant differences in the mean number of ovules per inflorescence between stations A and B, and station C ( $L_{A,B} = 1.699$ ,  $Sig\ 2-tailed = 0.950$ , mean difference = 9.893,  $L_{A,C} = 1.287$ ,  $Sig\ 2-tailed = 0.004$ , mean difference = 31.734 and  $L_{B,C} = 1.265$ ,  $Sig\ 2-tailed = 0.006$ , mean difference = 21.841) (**Figure 4**). The results suggest that there is a resource limitation at station C.



**Figure 4** Mean number with sd error bar of ovules per flower of pollination treatments at stations A and B (inside National Park) and station C (outside).



**Figure 5** The effect of supplementary hand-provided pollen on mean seeds/ovules ratios with sd error bar.

T-tests were conducted to detect differences between hand- and open-pollinated inflorescences in the 3 different studied locations. In stations A and B, the number of seed sets is significantly higher in hand-pollinated inflorescences than in open-pollinated inflorescences (**Figure 5**). T-tests detected significant differences between hand- and open-pollinated inflorescences on mean S/O ratios in station A and station B ( $T\text{-value} = 2.802$ ,  $Sig\ 2\text{-tailed} = 0.012$  and  $T\text{-value} = 2.524$ ,  $Sig\ 2\text{-tailed} = 0.021$  respectively).

The results suggest that there is a pollen limitation at stations A and B. Hand pollination significantly increased the mean number of initiated seeds by 95 %. However, at station C, there was no significant difference in the S/O ratios between open- and hand-pollinated treatments ( $T = 0.410$ , *Sig 2-tailed* = 0.550), indicating that there is no pollen limitation at this station.

## DISCUSSION AND CONCLUSIONS

The data suggested that station C was resource limited, while stations A and B were not resource limited (**Figure 4**). Poor humus and higher erosion are thought to be causes of resource limitation at station C. Station C is located next to the road on one side and on the edge flanking a stream on the other side. However, the habitat of station C or the surrounding area is possibly favored by the pollinators, *Trigona* sp.

The results also showed that there was pollen limitation at stations A and B (**Figure 5**). A possibility could be due to lack of pollinators. Stingless bees, *Trigona* sp., were the only pollinator observed at station C. *Trigona* bees are social bees which make nests on tree trunks in an uncrowded underground area [14]. These long-tongued shade-loving bees fly swiftly near the ground and traplined *E. littoralis* flowers. Stations A and B, are in secondary and intermediate disturbed forests respectively that are possibly not favored by pollinators. The nature of the surrounding habitat of stations A and B, unlike station C, is dense forest on the ground with many herbaceous plant species competing for light. Birds or spiderhunters were not found visiting the plants during our time of study. However, spiderhunters were found to be pollinators of *E. elatior* in a different area in Khao Nan National Park, about 17 km away from the study site. They are also reported to be pollinators for three species of *Etilingera*, i.e. *E. aff. metriocheilos*, *E. punicea* and *E. aff. brevilaris* in Borneo [5]. In addition, ants are found to be nectar thieves. The ants cover the whole inflorescence with soil, making it impossible for other pollinators to reach the flower's reproductive organs.

The pollination guild of *E. littoralis* could be assigned to Halictid-pollinated guild that comprises small, 9.8 - 10.4 mm in length and 2.8 - 3.1 width, bee pollinators [5]. When visiting the flowers, *Trigona* sp. landed on the lip near the anther and walked shortly into the corolla tube. It forages for pollen as a source of food from one flower to another. The pollen grains that are deposited on its head and hind legs, are the promoting source of cross pollination. This suggests that the morphology of the flower limits the type of pollinator. Fruit maturation takes about 4 months after pollination. Hard and thick shelled fruits of *E. littoralis* are usually buried under the soil, and are



therefore difficult to spot. We suspect that the fruits are sources of food for small rodents, seed dispersal vectors of the plant species.

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## บทคัดย่อ

ณภัทร กิตติพินังกุล และ ฉัตรชัย งามเรียบสกุล

ปัจจัยจำกัดของเรณูและพาหะถ่ายเรณู ต่อการเจริญของเมล็ดในปูดคางคก (*Etilingera littoralis*)  
ในพื้นที่ลุ่มน้ำคลองกลาย อุทยานแห่งชาติเขานัน

การศึกษายปัจจัยจำกัดของเรณูและพาหะถ่ายเรณูในปูดคางคก (*Etilingera littoralis* (J. König) Giseke) บริเวณพื้นที่ลุ่มน้ำคลองกลาย โดยการถ่ายเรณูด้วยมือ และการถ่ายเรณูตามธรรมชาติ จำนวน 3 สถานี คือ บริเวณภายในอุทยานแห่งชาติเขานัน นครศรีธรรมราช 2 สถานี (สถานี A และ B) และ ภายนอกอุทยานฯ 1 สถานี (สถานี C) สํารวจพบเพียงชันโรงชนิดเดียว (*Trigona* sp.) เป็นพาหะถ่ายเรณูที่สถานี C พบปัจจัยจำกัดของเรณู เมื่อเปรียบเทียบการถ่ายเรณูด้วยมือและการถ่ายเรณูตามธรรมชาติ สัดส่วนเมล็ดต่อออวูลจากการถ่ายเรณูด้วยมือ มากกว่าสัดส่วนเมล็ดต่อออวูลจากการถ่ายเรณูตามธรรมชาติ ที่ความแตกต่างอย่างมีนัยสำคัญ ในสถานี A ( $T = 2.802$ ,  $Sig$  2-tailed = 0.012) และ B ( $T = 2.524$ ,  $Sig$  2-tailed = 0.021) สถานี A (ค่าความแตกต่างเฉลี่ย = 0.547) มีปัจจัยจำกัดของเรณู มากกว่าสถานี B (ค่าความแตกต่างเฉลี่ย = 0.501) เล็กน้อย ส่วนสถานี C ไม่พบปัจจัยจำกัดของเรณู ( $T = 0.410$ ,  $Sig$  2-tailed = 0.550) เนื่องจากมีพาหะถ่ายเรณูตามธรรมชาติ คือ ชันโรง ทำให้การถ่ายเรณูตามธรรมชาติมีการเจริญของเมล็ดใกล้เคียงกันกับการถ่ายเรณูด้วยมือ อย่างไรก็ตามพบว่าสถานี C มีปัจจัยจำกัดของทรัพยากรจากการเปรียบเทียบจำนวนออวูลเฉลี่ยต่อดอก (ค่าความแตกต่างเฉลี่ย A,B = 9.893, ค่าความแตกต่างเฉลี่ย A,C = 31.734 และ ค่าความแตกต่างเฉลี่ย B,C = 21.841)