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THE POLLINATION BIOLOGY AND BREEDING SYSTEM OF *NEPENTHES MACFARLANEI* (NEPENTHACEAE)

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CHUA, L. S. L. 2000. The pollination biology and breeding system of *Nepenthes macfarlanei* (Nepenthaceae). *Nepenthes macfarlanei* has a xenogamous breeding system and exhibits a generalist entomophilous pollination syndrome. Potential pollinators include members of Diptera (Calliphoridae, Muscidae and Syrphidae). Low frequency of insect visits could be a limiting factor to successful pollination, but the species may be able to offset the limitation by employing strategies such as long periods of stigmata receptivity and synchronous receptivity in the pistillate inflorescence. Stigmata receptivity was visually observed to occur for about 30 days but the rate of pollination success was highest during the first two weeks of receptivity.

Keywords : Flower morphology - flower visitors - Diptera – pollination - xerogamy

CHUA, L. S. L. 2000. Biologi pendebungaan dan sistem pembiakan *Nepenthes macfarlanei* (Nepenthaceae). Pembiakan *Nepenthes macfarlanei* ialah jenis pembiakan luar dan spesies ini diperhatikan mengamalkan pendebungaan serangga. Pendebunga-pendebunganya tidak spesifik dan antara serangga yang mungkin memainkan peranan penting dalam pendebungaan ialah Diptera (Calliphoridae, Muscidae dan Syrphidae). Frekuensi lawatan serangga yang rendah mungkin merupakan penghalang kepada kejayaan pendebungaan. Namun demikian ia dapat mengatasi masalah ini dengan melanjutkan jangkamasa kepekaan stigmanya serta memastikan semua stigma pada jambak bunga peka pada masa yang sama panjang. Walaupun stigma diperhatikan peka selama 30 hari, kejayaan pendebungaan adalah paling tinggi dalam dua minggu yang pertama.

Introduction

The genus *Nepenthes* is dioecious. Kaul (1982), Adam *et al.* (1989), Kato (1993), Moran (1993), Phillipps and Lamb (1996), and Adam (1998) have suggested insects as pollinating agents based largely on the presence of nectar and foetid odour in the flowers and the regular encounter of insects on the inflorescences. Adam *et al.* (1989) recorded Diptera as the most frequent visitors on the flowers of *Nepenthes* × *kinabaluensis*, *N. villosa* and *N. rajah*, while Hymenoptera were most frequently seen visiting the flowers of *N. reinwardtiana* and *N.* aff. *fusca*. Other observed visitors were from Coleoptera and Lepidoptera and from the family Formicidae. Diptera (*Stomorhina* spp.) and Lepidoptera (*Herpetogramma* sp., *Pleuroptya* sp., *Pagyda* sp., *Ambia* sp. and *Pycnarmon* sp.) have been recorded on *N. gracilis* (Kato 1993). Most of the visits took place between early evening and midnight, the time coinciding with maximum nectar production (Kato 1993).

Chrysomelid beetles were the most frequent visitors to *N. rafflesiana* (Moran 1993), while small flies were frequent on *N. rajah* (Phillipps & Lamb 1996). This study provides information on the pollination syndrome and breeding system of *N. macfarlanei*, a montane species endemic to Peninsular Malaysia.

Materials and methods

Geographic and physical details of the study site, the weather patterns and the floral biology observation methods employed on the sampled population at Mt. Purun, Genting Highlands, Peninsular Malaysia, are given in Chua (2000). Seven pistillate inflorescences from five plants and seven staminate inflorescences from six plants were observed. These inflorescences were observed for fifteen days from 30 December 1993 to 22 April 1994, between 0900 and 1600 h, for time, frequency and duration of insect visits. Daily observation and trapping were conducted for four days from 12 to 15 January 1994 and the remaining eleven days were staggered up to 22 April 1994. Direct observations were made for foraging and feeding behaviours of flower visitors and the mode of pollen transfer. To determine night visitors, observations were also carried out for seven consecutive nights, from 1900 to 0700 h. Samples of flower visitors were captured for species determination and they were examined under the light microscope for the presence of pollen grains (scored + or 0).

Pollination manipulations were done to determine the breeding system of this species. Pistillate inflorescences bearing mature buds were isolated using nylon bags of 0.2-mm mesh size. Treatments used, each having five inflorescence replicates, were open pollination, control bagging and hand pollination. In open pollination, the selected inflorescences were not bagged, while in control bagging the inflorescences were bagged, but not manipulated. Inflorescences selected for hand pollination were bagged and their flowers were hand-pollinated with pollen grains from freshly dehisced anthers. Where possible, different plants were selected as replicates for a treatment, but occasionally a plant may be used more than once for the same treatment. In such cases, different inflorescences in the plant were used as replicates for the treatments. Buds in the pistillate inflorescence mature synchronously. The treatments were staggered throughout 17 months because it was not possible to have, at a single period of time, 15 inflorescences in the sampled population for pollination work.

Upon reaching receptivity, the stigmatic surface remained visibly unchanged for about three weeks (Chua 2000). In an experiment to determine whether the stigma is receptive to pollen transfer throughout this duration, between 18 and 20 buds in an inflorescence were randomly selected for a pollen application after 14, 21, 28 and 35 days and a control with no pollen application. Five inflorescences were used in this experiment. The treatments were staggered throughout 17 months and hand pollination was done around mid-day. Fruit set was scored and at maturity, the number of mature seeds was counted. The results were analysed using single factor ANOVA (model I) with unequal number of replicates (Sokal & Rohlf 1995).

Results

Flower morphology

The flower morphology of *N. macfarlanei* is designed to facilitate insect visits. At anthesis, the tepals, where nectar production is concentrated, are fully reflexed, providing a convenient landing and feeding platform for insects to forage. The diameter of the flower at this stage measured up to 1 cm. In the staminate flower, the anthers are held well above the tepals by an elongated androphore, while in the pistillate flower, the pistil, which is without style, is about 0.5 cm in height. This species has a typical dish-shaped flower. In the pistillate inflorescence, the synchronous receptivity and close arrangement of the flowers enabled the flower visitors to forage on as many flowers as possible. Similar floral morphology has been documented in other *Nepenthes* sp. (Danser 1928, Jebb & Cheek 1997).

Flower visitors

During the fifteen-day observation period, the total number of visits recorded on both pistillate and staminate inflorescences was 21 (Table 1). The staminate inflorescences had fifteen visits which were spread over ten days. All staminate inflorescences were visited at least once during the observation period but none was visited daily by flower visitors. Out of the fifteen visits, eleven were made by potential pollinators. The pistillate inflorescences had six visits; these visits were spread from day two to five, after which there were no longer any visits, although there were still display of floral rewards (Table 1). Like its opposite sex, the pistillate inflorescences were visited at least once during the observation period but none were visited daily by flower visitors. Out of the six visits, five were made by potential pollinators. This timing of visits coincided with the anthesis period in the sample population, which peaked during the month of January 1994 (Chua 2000). The absence of flower visitors from the pistillate inflorescences during the rest of the observation days can be attributed to the lower number of opened flowers.

The staminate inflorescences were visited by a variety of flower visitors, ranging from flies, wasps, bees and plant hoppers to ants, cockroaches, spiders and crabs (Table 1). Formicidae (ants) were the most frequent visitor but in this study their number of visits was not recorded as their foraging behaviour and the dioecious nature of *N. macfarlanei* do not make them a potential pollinator. Diptera was the second most common visitor; members from Muscidae (brown houseflies), Syrphidae (flower flies) and Calliphoridae (carrion flies) made one, four and one visit respectively, totalling six visits or 40% of the total number of visits. Halictidae (bees) made three visits, two of which took place on the same day, while Dictyopharidae (plant hopper) and Tiiphidae (tiiphid wasp) made one visit each (Table 1). Although cockroaches, spiders and crabs visited the flowers, they are unlikely to be potential pollinators.

Table 1. The frequency and number of flower visitors to the staminate and pistillate flowers of *Nepenthes macfarlanei* during a fifteen-day observation period from 30 December 1993 to 22 April 1994. (C* = Calliphoridae, D = Dictyopharidae, H = Halictidae, M* = Muscidae, S*= Syrphidae, T = Tiiphidae, Cb = crab, Ch = cockcroach, Mo = mosquito, Sr = spider; the number indicates the number of visits made on that day)

		No. of observation days															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Staminate	Flower visitors	1C	1M	2H	1Sr	3S	0	0	0	0	0	1Cb, 1D	1T	2Ch	1H	1S	15
	Potential pollinators	1C	1M	2H	0	3S	0	0	0	0	0	1D	1T	0	1H	1S	11
Pistillate	Flower visitors	0	3M	1M	1Mo	1M	0	0	0	0	0	0	0	0	0	0	6
	Potential pollinators	0	3M	1M	0	1M	0	0	0	0	0	0	0	0	0	0	5

*Members of Diptera group.

In comparison, only ants, Muscidae fly and mosquitoes visited the pistillate inflorescences (Table 1). Once again, ants were the most frequent visitor. The second most common visitor was Muscidae flies, which made five visits during the observation period. It is interesting to note there were no visits made by syrphid flies, carrion flies, halictid bees, plant hoppers and tiiphid wasps. In addition, muscid flies seemed more attracted to the pistillate flowers than the staminate flowers. No pollen loads were, however, observed on the flies captured from the pistillate flowers. Lepidoptera (butterflies and moths) did not visit the flowers of both sexes although they were observed in the area on certain days and were less than 2 m away from mature flowers.

Both the pistillate and staminate inflorescences attracted only one insect at a single time. The synchronous anthesis and display of floral rewards by the pistillate flowers in an inflorescence were not observed to enhance the number or frequency of visits by potential pollinators. The highest number of visits made in a day to a single pistillate inflorescence in the sampled population was three. These visits took place on 12 January 1994 at 1300, 1330 and 1337 h.

Floral visits to the pistillate and staminate inflorescences were recorded from 0930 to 1650 h; 41% of the total number of visits occurred before 1210 h while another 47% took place between 1300 and 1400 h, and the remaining 12% took place after 1400 h. For the pistillate inflorescences, five out of six visits took place between 1300 and 1337 h. Visits to the anthers coincided with anthesis time, which occurred between 0630 and 1700 h (Chua 2000). At night, only cockroaches were seen visiting the flowers.

The visits lasted between 3 and 40 min. Most insects aimed for the nectar; a few such as carrion flies foraged in addition, the pollen grains. The insects did an unhurried and thorough crawl through the mature flowers. In the staminate inflorescence, the anthers, although more widely spaced, were smaller in size compared to many insect visitors, and the visitors, in their foraging scramble along the tepals, collected clumps of pollen grains on their legs, thorax and abdomen. Many of these clumps were large and visible. In the pistillate inflorescence, the close arrangement of the flowers and the insect's scramble over the ovaries, consequently brushing against more than one flower, facilitated pollen deposition onto the stigma. Little of the effort, however, was likely to be successful as the densely adpressed hairs on the ovaries increased pollen loss.

Seed set from pollination experiments

The number of mature seeds was significantly different for fruits that developed in control bagging, open and hand pollination (Table 2; $p \leq 0.05$). There was, however, no significant difference in the mean number of mature seeds developing from open and hand pollination. Flowers in the control bagging treatment died soon after the loss of receptivity, while 57.3% of the sampled flowers in open pollination had successful fruit set. In hand pollination, 60% of the treated flowers developed into mature fruits.

Multiple range test results showed that the timing of pollen application was highly significant (Table 3; p≥0.05). The number of mature seed set following pollination at day 14 was the highest among all treatments. Although means for day 21 and 28 treatments were less compared to day 14, their small numbers are sufficient to indicate that the stigmata at 21 and 28 days were still receptive to pollen grains. This receptivity, however, was lost by day 35 (Table 3, Figure 1).

Table 2. *t*-test (LSD) for mean number of mature seeds of *N. macfarlanei* developing from control bagging, open and hand pollination. (Means with the same letter are not significantly different at $p \le 0.05$, df = 49. Results are simplified for display.)

Treatment	N	Mean No. of mature seeds
Open pollination	20	92.50 a
Hand pollination	10	78.10 a
Control bagging	20	0 b

Table 3. Waller-Duncan *K*-ratio *t*-test for mean number of mature seeds of *N. macfarlanei* developing from flowers pollinated at day 14, 21, 28, 35 and control with no pollen application. (Means with the same letter are not significantly different at $p \le 0.05$, df = 96.)

Day	N	Mean No. of mature seeds
14	20	64.40 a
21	20	23.75 b
28	19	26.32 b
35	20	0.7 c
Control	18	0.61 c

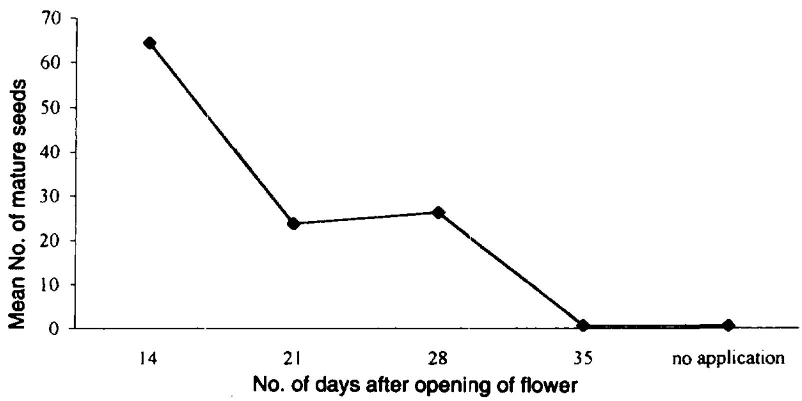

Figure 1. Mean number of mature seeds for flowers hand-polinated at 14, 21, 28 and 35 days after flower opening including a control with no pollen application

Discussion

Pollination syndrome

Floral rewards in the flowers of *N. macfarlanei*, aided by an unsophisticated dish-shaped perianth, attracted many different types of insects, as well as cockroaches, arachnids and crabs. Flies were more attracted to the flowers than other groups of insects. Ants are considered nectar robbers and not pollinators (Beattie *et al.* 1984, Kato 1993). Wind is ruled out as the primary pollinating agent as the pollen grains are disposed of in large clumps.

The present observations add further credence to the suggestion that the genus exhibits a generalist entomophilous syndrome. Diverse groups of insects have been implicated and examples are moths (Lepidoptera) in *N. gracilis* (Kato 1993) and chrysomelid beetles (Coleoptera) in *N. rafflesiana* (Moran 1993). Coincidental timing of floral visits and foraging behaviour of muscid flies on the pistillate and staminate flowers seemed to suggest that they may play a role in the pollination of *N. macfarlanei* although specimens captured from pistillate flowers during the observation days had no pollen load.

Free (1960), Frankie *et al.* (1976) and Bawa (1977) have shown that many of the small generalist insects have restricted foraging ranges and that they often returned to the same resource patch. This has important implications for *N. macfarlanei*, for long distance gene flow through pollen is not likely to happen between populations placed further apart than the size of the patch within which the potential pollinators forage. This is particularly relevant to populations on isolated summits and summits in a large massif. The presence of hybrids near their parent species and not in localities elsewhere is also indicative that there are physical barriers to long distance gene flow. This is perhaps one of the reasons why many *Nepenthes* sp. are restricted to a single locality (Jebb & Cheek 1997).

Primack (1985) and Stratton (1989) have found that, in general, flowers in the cloud forests have a longer life span than flowers in the lowland rain forests. This is particularly true for *N. macfarlanei* whose pistillate flowers exhibit a long period of receptivity. However, its rate of pollination success is likely to be highest during the first two weeks of stigmata receptivity. This feature is perhaps a strategy to increase competitiveness for pollinators and opportunities for mating. The low frequency of visits observed in this study suggests that keen competition for pollinators exists at certain periods during the flowering season. To reduce such barriers, adaptive strategies such as having a long flowering season, a pistillate bias sex ratio and a staminate bias flower ratio in the population are used (Chua 2000).

Breeding system

The complete failure of control bagging flowers to set fruit indicates that this species is xenogamous. This breeding system, believed to be consistent in Nepenthaceae, is reflected in the ease by which *Nepenthes* hybridises either in nature or in artificial environment (Danser 1928, Schlauer 1994, Phillipps & Lamb 1996, Jebb & Cheek 1997).

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