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THE FLOWERING PHENOLOGY AND FLORAL BIOLOGY OF *NEPENTHES MACFARLANEI* (NEPENTHACEAE) FROM MT. PURUN, PENINSULAR MALAYSIA

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CHUA, L. S. L. 2000. The flowering phenology and floral biology of *Nepenthes macfarlanei* (Nepenthaceae) from Mt. Purun, Peninsular Malaysia. The phenology and flower development from opening to senescence and fruit formation was observed in 44 mature plants of *Nepenthes macfarlanei* Hemsl. at Mt. Purun, Pahang, Peninsular Malaysia. These plants flowered continuously throughout the 23-month phenological observation. Of these 73% plants were pistillate plants while the remaining 27% were staminate plants. The sampled population produced 1588 pistillate and 3183 staminate flowers during the period. There was a distinct peak period where 57.7% of the pistillate flowers and 74.6% of staminate flowers reached anthesis synchronously. The staminate and pistillate inflorescences required 4–7 weeks after emergence from leaf sheaths for the first bud to reach anthesis. Stigma receptivity occurred at any time of the day while anther dehiscence occurred between 0630 and 1700 hours. Fertilised flowers developed into mature fruits in 20–26 weeks. The mature fruit produced an average of 136 seeds but only two-thirds of the seeds were mature. The sampled population was estimated to produce approximately 200 000 mature seeds during the phenological period. There was a high mortality of pistillate plants towards the end of the period.

Key words: Peninsular Malaysia - *Nepenthes* - floral biology - phenology - sex ratio - mortality - seed production

CHUA, L. S. L. 2000. Fenologi pembungaan dan biologi bunga bagi *Nepenthes macfarlanei* (Nepenthaceae) dari Gunung Purun, Semenanjung Malaysia. Fenologi dan perkembangan bunga daripada peringkat awal sehingga kematangan buah telah diperhatikan dalam 44 individu matang *Nepenthes macfarlanei* Hemsl. di Gunung Purun, Pahang, Semenanjung Malaysia. Sampel populasi ini berbunga di sepanjang tempoh fenologi iaitu selama 23 bulan. Daripada bilangan ini, 73% ialah jenis betina dan 27% ialah jenis jantan. Dalam tempoh ini, sampel populasi menghasilkan 1588 bunga betina dan 3183 bunga jantan. Terdapat satu tempoh apabila 57.7% bunga betina dan 74.6% bunga jantan mencapai antesis serentak. Jambak bunga betina dan jantan memerlukan 4–7 minggu selepas wujud daripada pelepah daun untuk kudup bunga pertama mencapai antesis. Kepekaan stigma boleh berlaku bila-bila masa sementara pemecahan anter berlaku antara jam 0630 sehingga 1700. Bunga yang telah disenyawakan memerlukan 20–26 minggu untuk berkembang menjadi buah. Buah matang menghasilkan purata 136 biji tetapi hanya dua pertiga daripada jumlah ini merupakan biji matang. Sampel populasi dianggarkan boleh menghasilkan kira-kira 200 000 biji matang dalam tempoh fenologi ini. Peratus kematian di kalangan individu betina adalah tinggi pada akhir tempoh fenologi.

Introduction

The genus *Nepenthes* is dioecious and the inflorescence is either a raceme or a panicle (Danser 1928). The inflorescence arises from the apex of the stem (Danser 1928, Kurata 1976). The staminate inflorescence has more flowers compared with the pistillate inflorescence; in *N. gracilis*, Kato (1993) recorded an average of 90 flowers in the staminate inflorescence compared with 52 flowers in the pistillate inflorescence. In some species, pistillate flowers mature synchronously (Danser 1928, Phillipps & Lamb 1996) while in others such as *N. gracilis*, they mature acropetally (Kato 1993). The flowering of *Nepenthes* in Peninsular Malaysia is thought to be seasonal (Holttum 1940). Insects are suspected to be pollinating agents based on the presence of nectaries and observations of flower visitors (Daumann 1930, Lim & Prakash 1973, Kaul 1982, Adam *et al.* 1989, Kato 1993, Adam 1998). Harms (1936) and later works have indicated that seeds are wind-dispersed. Many of the previous reports on the floral biology of *Nepenthes* have been based on static observations (Danser 1928, Harms 1936, Kurata 1976, Kaul 1982, Phillipps & Lamb 1996, Adam 1998) while the flowering phenology for most species is scarcely known. This study provides information on the flowering phenology and floral biology of *Nepenthes macfarlanei*, a species endemic to the montane forests of Peninsular Malaysia.

Materials and methods

Phenological and floral biology observations were conducted at Mt. Purun, Genting Highlands (101° 47'E, 3° 25'N; 2011 m above sea-level). The parent rock is granitic (Tjia 1988) and the soil is peaty gley podzols, blanketed with peat (Whitmore & Burnham 1969). The vegetation is montane ericaceous forest, characterised by *Leptospermum amboinense, Dacrydium comosum, Rhododendron malayanum, Vaccinium viscifolium, Exbucklandia populnea* and *Pandanus klossii* (Stone 1981, Chua 1995). The mean monthly temperature and rainfall at Mt. Purun are 19.7°C and 294.5 mm respectively (Tan, Resort World Sdn. Bhd., personal communication).

Weekly phenological observations were conducted from August 1993 to July 1995. All mature plants in a 0.64-ha ecological plot were monitored for flowering activity. The parameters recorded were the number of plants flowering, the number of inflorescences produced per plant, the corresponding number of staminate and pistillate flowers and the associated floral changes from bud stage to maturity and fruiting. Floral induction and early bud development were not studied because they required destructive sampling which meant that there would be less flowers available for phenological and floral biology observations and for subsequent pollination work (not reported here). For a more detailed floral biology study, selected staminate and pistillate flowers were observed for time of anthesis (anthesis here is defined as anther dehiscence in the staminate flowers and stigma receptivity in the pistillate flowers). The anthesis study was conducted for 69 days, between 30 December 1993 and 9 March 1994. The staminate flowers were observed at 2-h intervals between 0700 and 1900 hours, while the development of pistillate flowers and fruits was observed at a weekly interval. Flowers were also observed for seven nights between 1900 to 0700 hours for anthesis. The total numbers of staminate and pistillate flowers observed for anthesis were 281 and 89 respectively. A Scanning Electron Microscope (Model JEOL) was used to scan pollen grains from dehisced anthers while standard anatomical procedures were used to determine ovule development (Johansen 1940).

Results

Out of the total 228 plants present in the ecological plot, 44 plants flowered during the 23-month phenological period. Of this number, 32 were pistillate plants and 12 were staminate plants. Twenty-three plants, all pistillate, however, died during the period while the remaining 21 plants prevailed. Towards the end of this period, 8 staminate plants stopped flowering. The sex ratio fluctuated from 1.9 pistillate plants : one staminate plant at the early part of the period to 2.3 pistillate plants: one staminate plant at the end of the period. The flower sex ratio, although showing great fluctuations throughout the period, remained mostly staminate bias (Figure 1, Table 1). The following results are based on data collected from 44 plants because they represented the total number that flowered during the phenological period.

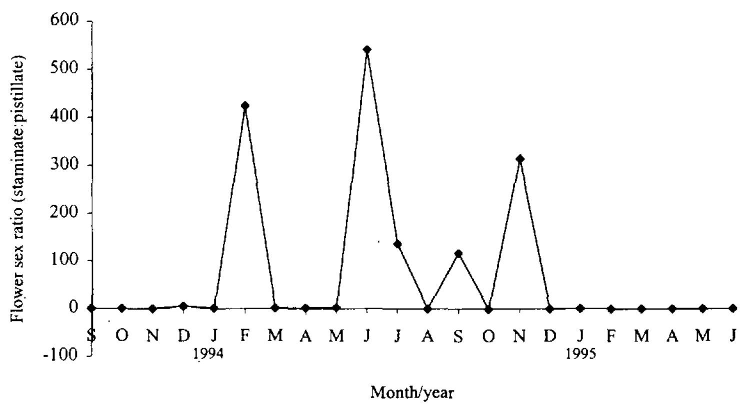

Figure 1. Fluctuation in the flower sex ratio for the sampled population at Mt. Purun during August 1993–July 1995

A pistillate plant produced an average of 1.5 inflorescences; there were, however, several plants that were able to produce up to 3 inflorescences. During the phenological period, 34 inflorescences bearing a total of 1588 flowers reached maturity while 19 others died without achieving maturity. There was no abortion of buds in the mature inflorescences. Immature inflorescences that died were those produced later by the plants or those produced towards the end of the phenological period. During this period, flowering in all pistillate plants showed two anthesis peaks with minor anthesis occurring outside these peaks (Figure 2). During the first peak (November 1993 to May 1994), 916 pistils from 12 plants became receptive. This number represented 57.7% of the total number of pistils reaching anthesis during the phenological period. During the second peak (November 1994 to March 1995), 448 pistils from 9 plants were receptive. Anthesis for the remaining 224 pistillate flowers were spread outside the peaks (Table 1).

Table 1. Number of pistillate and staminate inflorescences and flowers at anthesis[1] and the changes in flower sex ratio in the sampled population at Mt. Purun during August 1993–July 1995

Year	Month	Pistillate No. inflorescences	No. flowers	Staminate No. inflorences	No. flowers	Flower sex ratio (S:P)[2]
1993	August	0	0	0	0	0
	September	0	0	0	0	0
	October	0	0	0	0	0
	November	0	0	0	0	0
	December	1	35(100)	1	198 (97.5)	5.66
1994	January	10	564(100)	5	591(100)	1.05
	February	0	0	4	424(99.8)	424
	March	2	93(100)	1	135(100)	1.45
	April	2	101(100)	1	162(99.4)	1.60
	May	3	123(100)	2	300 (95)	2.44
	June	0	0	5	541(100)	541
	July	0	0	1	137(100)	137
	August	1	42(100)	0	0	-0.02
	September	0	0	1	118(100)	118
	October	1	36(100)	0	0	-0.03
	November	0	0	2	314(100)	314
	December	6	243(100)	0	0	-0.004
1995	January	3	151(100)	2	163(22.7)	1.08
	February	1	54(100)	0	0	-0.02
	March	0	0	0	0	0
	April	0	0	0	0	0
	May	0	0	0	0	0
	June	0	0	0	0	0
	July	0	0	0	0	0

[1]Number in parentheses refers to the percentage of mature flowers.
[2]Staminate: pistillate ratio.

A staminate plant produced an average of 2.2 inflorescences; there was, however, one that produced 5 inflorescences. During the observation period, 26 inflorescences, bearing a total of 3183 staminate flowers, were produced. Unlike the pistillate plants where there was abortion in young inflorescences, all staminate inflorescences produced during this period developed to maturity stage. However, only

93% of the total number of buds attained anthesis. Buds failing to reach anthesis were either positioned at the distal end of the inflorescence or were in inflorescences produced later in the season. Anthesis for staminate flowers occurred mainly between December 1993 and August 1994 (Figure 2). During this time, 2208 mature flowers from 18 inflorescences achieved anthesis. This represented 74.6% of the total number of mature flowers produced. The remaining 753 staminate flowers had anthesis outside this period (Table 1).

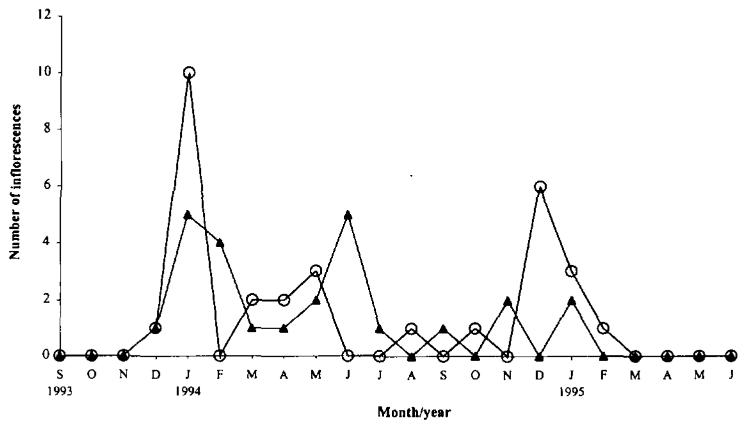

Figure 2. The number of female (open circle) and male inflorescences (solid triangle) at anthesis in the sampled population at Mt. Purun during August 1995–July 1995

After emergence from the leaf sheaths, the pistillate inflorescence required a further 5.3 ± 1.68 s.d. weeks for the buds to reach anthesis. The average number of pistillate flowers per inflorescence, at 46.7 ±15.19, was less than half the average number of staminate flowers per inflorescence (Table 2). Anthesis, which coincided with the opening of tepals, may occur at any time of the day and during anthesis, the stigmatic surface is green and glistens with nectar. The stigmatic surface remains green and glistening for about three weeks.

Table 2. Summary of floral biology data for the sampled population at Mt. Purun from August 1993 to July 1995

	Pistillate	Staminate
Total sample size	32	12
Sample size at the end of observation period	9	12
Total No. of mature inflorescences	34	26
Mortality of inflorescences	19	0
Total No. of mature flowers	1588	3183
Average No. of inflorescences per plant	1.5	2.2
Average No. of flowers per inflorescence	46.7 ± 15.19	122.4 ± 32.97
Anthesis time	Throughout the day	0630–1700 hours
Average No. of mature seeds per fruit	90.6 ± 14.54	-
Average No. of weeks to fruit maturity	23.2 ± 3.24	-

Anatomical sections of the ovules taken during the early stages of anthesis, i.e. when the tepals are semi-reflexed, showed no detectable presence of functional megaspore mother cells (MMC). Mitosis was apparent in some archesporial cells (Figure 3). MMCs with clearly defined inner and outer integument layers and nucellus tissue were present at the fully reflexed tepal stage (Figure 4). Nuclear activity, related to meiosis, was clearly evident in some of the MMCs although embryo sacs could not be detected in any of the sections (Figure 5). It was observed that ovules in a single ovary had different stages of megaspore development; at the height of anthesis, some ovules had functional MMCs while in others, they were just developing. Such a synchronous development of ovules has also been reported for *N. gracilis* (Lim & Prakash 1973).

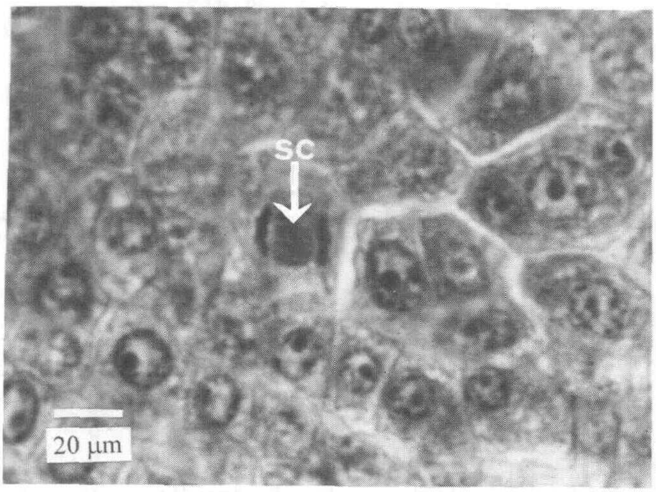

Figure 3. Longitudinal section of an ovule showing mitotic activity in the sporogenous cells (sc). Magnification × 100.

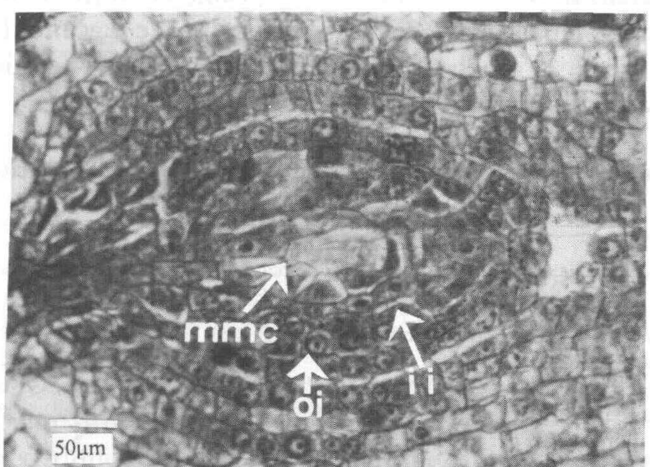

Figure 4. Longitudinal section of an ovule showing a functional megaspore mother cell (mmc) bearing nucellus tissue and inner (ii) and outer integument (oi) layers. Magnification × 40.

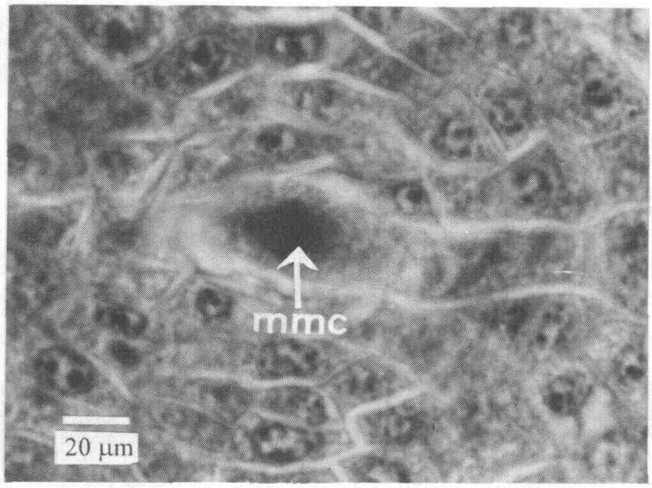

Figure 5. Longitudinal section of an ovule showing nuclear activity, presumably related to meiosis, in a megaspore mother cell (mmc). Magnification × 100.

After emergence from the leaf sheaths, the staminate inflorescence required a further 5.4 ± 1.14 s.d. weeks for the first bud to reach anthesis. Thereafter, the inflorescence displayed a daily average of 5.7 ± 5.4 s.d. mature flowers. The average number of staminate flowers per inflorescence was 122.4 ± 32.97 (Table 2). The large difference in inflorescence size between the staminate and the pistillate inflorescences is a reliable field character that may be used to identify the sex of the plant. This character was already visible whilst enclosed in leaf sheaths. The colours of the androphore and anthers at anthesis were light green and yellow respectively and nectar production was confined to the tepals. Anther dehiscence took place between 0630 and 1700 hours and there was no dehiscence at night unlike that reported for *N. gracilis* (Lim & Prakash 1973). The yellow pollen grains, adhering in tetrahedral tetrads, are covered with ovate-triangular spines and are morphologically similar to other *Nepenthes* spp. (Erdtman 1972, Kaul 1982, Adam 1998). Several screenings with SEM showed that each grain in the tetrad was able to germinate pollen tube (Figures 6a & 6b). This showed that the presence of other grains within the tetrad does not inhibit pollen tube germination.

The fruit pods of *N. macfarlanei* required an average of 23.2 ± 3.24 s.d. weeks to reach maturity. A mature fruit produced an average number of 136 ± 14.54 s.d. seeds. However, only two-thirds of the seeds were mature. Based on an average number of 1.5 inflorescences per plant and 46.7 flowers per inflorescence, a plant is estimated to produce about 9500 seeds during the phenological period, of which about 6300 would be mature seeds. For the same period, if all 32 plants produced at the same average number of inflorescences and flowers, there would be *300 000* seeds available at the sampling site, of which two-thirds (i.e. 200 000) would be mature seeds. Large seed production was noted in other species of *Nepenthes* (Danser 1928, Phillips & Lamb 1988) but not in *N. lowii* (Kaul 1982).

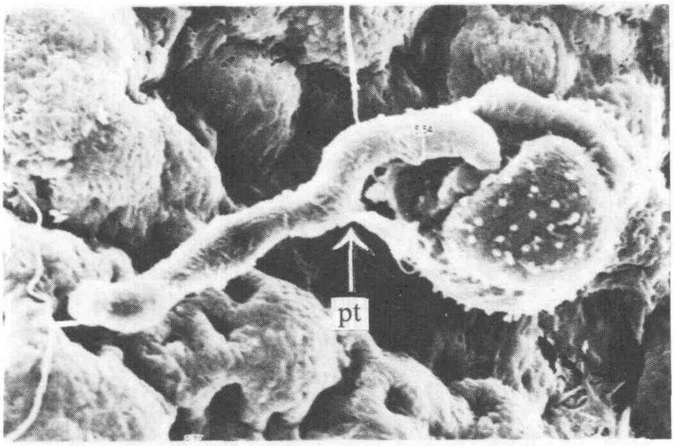

Figure 6a. Pollen tube (pt) development. Magnification × 1500.

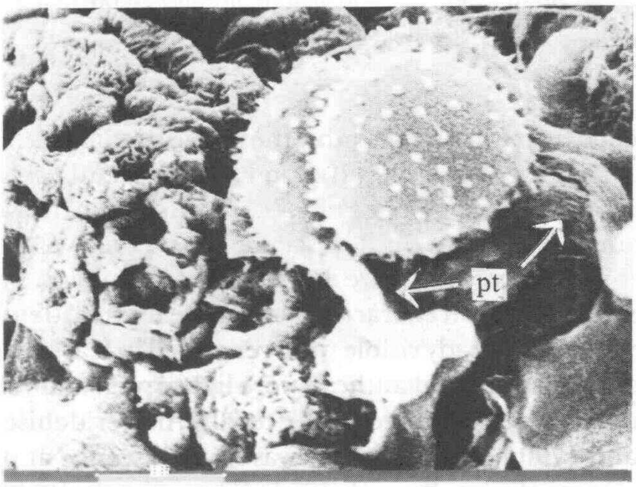

Figure 6b. The development of more than one pollen tube (pt) within a pollen tetrad. Magnification × 2000.

Discussion

The presence of staminate and pistillate flowers throughout the 23-month observation period indicates that *N. macfarlanei* flowers continuously. Although the period may not be sufficient to confirm this, the presence of fruits prior to August 1993 indicated past flowering activity. The continuous flowering activity displayed by *N. macfarlanei* at this site can be confused with Holttum's (1940) statement that flowering in Malayan *Nepenthes* is seasonal. The flowering season of *N. macfarlanei* was much longer than the period implied by Holttum (1940) in his use of the term 'seasonality'. However, the high mortality of pistillate plants towards the end of the phenological period suggests that peak flowering in the population was drawing to a close. In order to avoid further confusion with the term 'seasonality', the flowering behaviour in *N. macfarlanei* at Mt. Purun is best described as gregarious within the population since there were distinct flowering peaks as well as sporadic flowering outside these peaks.

Elsewhere in Peninsular Malaysia, *N. macfarlanei* flowered only sporadically, indicating that populations in different localities have variable flowering periods. This is drawn from brief observations on a population at Cameron Highlands (101° 25' E, 4° 30' N; 2070 m above sea-level). Observations on other species such as *N. ramispina* and *N. sanguinea,* which co-habit in the plot area at Mt. Purun, gave varying results. *Nepenthes ramispina* flowered only sporadically while *N. sanguinea* did not flower during the observation period, probably because many of the plants were immature.

There is an apparent flowering rhythm in both sexes during the phenological period. The coincidental timing of anthesis between the staminate and pistillate flowers where, during that time, a very large percentage of the total number of flowers produced by the sampled population had reached anthesis, indicates near absolute intra population synchrony. What triggers the timing of flowering in this population remains largely unknown. Physical and biotic factors affecting flowering initiation such as changes in microclimate and pollinator availability have been purported by many pollination biologists (Wong 1983, Reich & Borchert 1984, Koptur *et al.* 1988,Wright & Cornejo 1990, Foster 1993, van Schaik *et al.* 1993) but their roles in the *N. macfarlanei* population remain elusive. The temperature and rainfall data acquired from secondary sources for Mt. Purun were insufficient to interpret the influence of microclimate on plant reproduction.

The plant sex ratio of the population at the time of sampling was pistillate bias. This ratio is uncommon in tropical dioecious species where a study on 23 such species showed that only 2 species, both belonging to the family Polygalaceae, demonstrated pistillate-dominance (Opler & Bawa 1978). For *N. macfarlanei*, the population's sex bias fluctuated during the flowering period as many of the pistillate plants died and some of the staminate plants stopped flowering. This fluctuation, however, did not change the bias of its flower sex ratio, which, although showing great fluctuations, remained staminate throughout the phenological period. Out of the three components contributing to the relative number of pistillate and staminate flowers in the population (Opler & Bawa 1978), the greatest contribution to such a ratio came from the number of flowers per inflorescence. The factors contributing to the high mortality among the pistillate plants are not clearly known at present. The author observed that there were no apparent attacks of pests or diseases at the sampled population during the observation period.

Major limiting factors to sexual reproduction detected in *N. macfarlanei* are the asynchronous development of ovules and embryos and the inconsistent frequency of visits by potential insect pollinators (Chua 1995). In order to optimise seed production, the species employs staminate bias sex ratios and the pistillate flower undergoes relatively long periods of stigma receptivity (Chua 1995). Although pollen grains are available throughout the flowering period, the availability peaks when a large number of pistillate flowers become receptive, thereby ensuring sufficient amount reaching the stigmata. In addition, all pollen grains within the tetrad are capable of producing pollen tubes. And in order to cope with the limitation of seed dispersal by wind, the species produces large amounts of seed. Such floral behaviour places high maternal cost onto the plant and population. During the 23-month period, 72% of the flowering pistillate members in the sampled population died after fruiting. Despite this setback, however, a large number of mature seed had been released. Physical barriers to germination are minimal since moisture is ever present in the wet upper montane forest. In addition, ecological sampling of the area showed an inverse-J population where at least 48% of the sampled population were young plants in rosette stage (Chua 1995).

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References

ADAM, J. H. 1998. Reproductive biology of Bornean *Nepenthes* (Nepenthaceae) species. *Journal of Tropical Forest Science* 10(4):456–471.

ADAM, J. H., WILCOCK, C. C. & SWAINE, M. D. 1989. Ecology and taxonomy of Bornean *Nepenthes*. *Tropical Biology Newsletter* 56:2–4.

CHUA, L. S. L. 1995. Conservation Studies with *Nepenthes macfarlanei* Hemsl. in Peninsular Malaysia. Ph.D. thesis, University of Bath. 200 pp.

DANSER, B. H. 1928. The Nepenthaceae of the Netherlands Indies. *Bulletin du Jardin Botanique de Buitenzorg* IX:249–438.

DAUMANN, E. 1930. Das Blutennektarium von *Nepenthes*. *Beihefte Botanischen Zentralblatt* 47:1–14.

ERDTMAN, G. 1972. *Pollen Morphology and Plant Taxonomy. Angiosperms (An Introduction to Palynology)*. Hafner Publishing Co., New York: 283–284.

FOSTER, R. B. 1993. Famine on Barro Colorado Island. Pp. 201–212 in Leigh E. G., Rand, A. S. & Windsor D. M. (Eds.) *The Ecology of a Neotropical Forest: Seasonal Rhythms and Long-term Changes.* Smithsonian Institute, Washington.

HARMS, H. 1936. *Nepenthaceae Engler-Prantl. Die Naturlichen Pflanzenfamilien*. 2 Auflage. Band 17b:728–765.

HOLTTUM, R. E. 1940. Malayan pitcher plants. *Malayan Nature Journal* 1:35–44.

JOHANSEN, D. A. 1940. *Plant Microtechnique.* First edition. McGraw-Hill, New York. 80 pp.

KATO, M. 1993. Floral biology of *Nepenthes gracilis* (Nepenthaceae) in Sumatra. *American Journal of Botany* 80:924–927.

KAUL, R. B. 1982. Floral and fruit morphology of *Nepenthes lowii* and *Nepenthes villosa*, montane carnivores of Borneo. *American Journal of Botany* 69:793–803.

KOPTUR, S., HARBER, W. A., FRANKIE, G. W. & BAKER, H. G. 1988. Phenological studies of shrub and treelet species in tropical cloud forests of Costa Rica. *Journal of Tropical Ecology* 4:323–346.

KURATA, S. 1976. *Nepenthes of Mount Kinabalu*. Sabah National Parks Publication No. 2. Sabah National Parks Trustee, Malaysia. 80 pp.

LIM, A. L. & PRAKASH, N. 1973. Life history of *Nepenthes gracilis*. *Malaysian Journal of Science* 2:45–53.

OPLER, P. A. & BAWA, K. S. 1978. Sex ratios in tropical forest trees. *Evolution* 32(4):812–821.

PHILLIPPS, A. & LAMB, A. 1996. *Pitcher Plants of Borneo*. Natural History Publications (Borneo) Sdn. Bhd., Royal Botanic Gardens, Kew and Malaysian Nature Society. 171 pp.

REICH, P. B. & BORCHERT, R. 1984. Water stress and tree phenology in a tropical dry forest in the lowlands of Costa Rica. *Journal of Ecology* 72:61–74.

STONE, B. C. 1981. The summit flora of Gunung Ulu Kali (Pahang, Malaysia). *Federated Museums Journal* 26 (Part 1). Museum Department, Kuala Lumpur. 157 pp.

TJIA, H. D. 1988. The physical setting. Pp. 1–19 in Cranbrook, E. (Ed.) *Key Environments — Malaysia*. Pergamon Press, London.

VAN SCHAIK, C. P., TERBORGH, J. W. & WRIGHT, S. J. 1993. The phenology of tropical forests: adaptive significance and consequences for primary consumers. *Annual Review of Ecology & Systematics* 24:353–377.

WHITMORE, T. C. & BURNHAM, C. P. 1969. The altitudinal sequence of forests and soils on granite near Kuala Lumpur. *Malayan Nature Journal* 22:99–118.

WONG, M. 1983. Understory phenology of the virgin and regenerating habitats in Pasoh Forest Reserve, Negeri Sembilan, West Malaysia. *Malaysian Forester* 46:197–223.

WRIGHT, S. J. & CORNEJO, F. H. 1990. Seasonal drought and the timing of flowering and leaf fall in a neotropical forest. Pp. 49–61 in Bawa, K. S. & Hadley, M. (Eds.) *Reproductive Ecology of Tropical Forest Plants*. UNESCO/Parthenon, Paris.