Origin of insect pollination

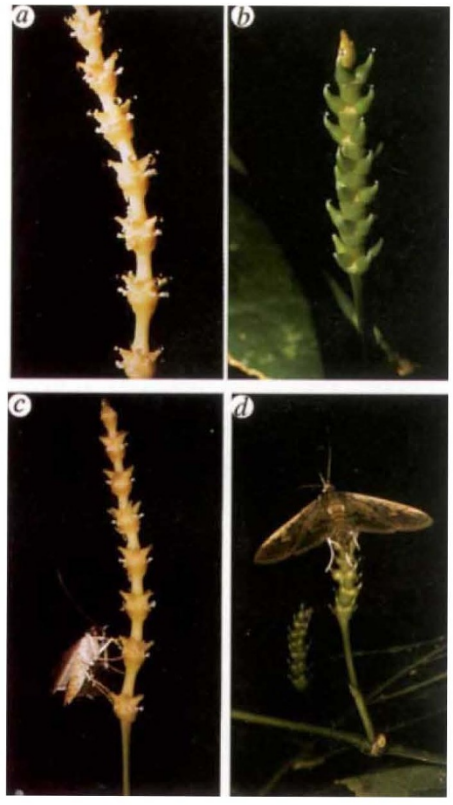
SIR — The Gnetales, a bizarre order of gymnosperms thought to be a sister group of angiosperms[1,2], comprise three distinct genera, *Ephedra, Welwitschia* and *Gnetum*; the first two are xerophytic and pollinated mainly by wind and partly by insects[3]. *Gnetum* is restricted to tropical rain forests and its pollination mechanism has not been definitely established. We have observed insect visits to a dioecious shrub. *Gnetum gnemon*, in a lowland mixed dipterocarp forest in Sarawak, Malaysia. Pollination droplets were secreted from ovules on female strobili and from sterile ovules on male strobili in the evening and were consumed by nectar-seeking moths of Pyralidae and Geometridae. Sticky pollen was attached on probosces and antennae of these moths. The moth attraction in *Gnetum* would have arisen from unspecialized entomophily, which is thought to be an original pollination system of early Gnetales and early angiosperms[4].

One hypothesis of the angiosperm diversification in the Cretaceous is the adoption of insect pollination[5]. Recent palaeobotanical evidence suggests that early Gnetales and early angiosperms co-occurred in mesic floodplain habitats and had similar vegetative morphology (small herbs or shrubs)[6], similar ecological tolerances and similar reproductive biology (flowers simple and pollinated by wind or unspecialized nectar-seeking insects)[4]. Thus, pollination systems of extant Gnetales may hold the key to the problem of angiosperm radiation. Among the three genera of the Gnetales, anemophily is prevalent in *Ephedra* and entomophily is known in only a few species of *Ephedra*[7] and rarely in *Welwitschia*[3]. *Gnetum* is an enigmatic genus: little is known of its pollination biology, although there are some morphological indications that point to entomophily[8].

In the Malayan region, 16 species of *Gnetum* are known; two are trees or shrubs and others are woody climbers. We studied the Floral biology of a dioecious shrub, *Gnetum gnemon* Linné var. *tenerum* Markgraf, in a lowland mixed dipterocarp forest in Lambir Hills National Park, Malaysia (4° 2′ N, 113° 50′ E, altitude 60 m). We observed Flowering throughout the year, but the flowering period of individual shrubs was less than 3 weeks. In preliminary observations made in the daytime between 21 and 30 November 1992, we found no flower visitors. We made night observations between 5 and 31 August 1993.

Ovules on female strobili and sterile ovules on male strobili secreted pollination droplets in the evening. Sequential sampling of droplets of pollinator-excluded strobili showed that droplet secretion started at about 18:00 h, and ceased by 21:00 h in male and by 20:00 h in female strobili, respectively. If droplets remained intact, the mean standing crop of a droplet increased to 0.164 in male and to 0.190 μl in female strobili at 21:00 h (*a*, *b* in the figure). We did not observe rapid withdrawal of droplets after pollination.

The droplets contained sugar, the concentration of which ranged from 3 to 13%, which is lower than that of an entomophilous *Ephedra* (72–80%)[7] but is higher than that of anemophilous conifers (for example, *Pinus*, 1.2%)[9]. The sugar concentration of the droplet was affected by the relative humidity of the surrounding air, which usually increased to more than 95% in the evening in the forest floor habitat. Nectar was also secreted among microsporangiphores on male strobili, although in very small amounts.



Strobili of *Gnetum gnemon* in the evening in a tropical rain forest in Sarawak. *a*, Sterile ovules emitting droplets (male strobilus); *b*, fertile ovules emitting droplets (female strobilus); *c*, a male strobilus visited by a pyralid moth, *Herpetogramma* sp.; *d*, a female strobilus visited by a pyralid moth, *Hedylepta* sp.

Male strobili secreting droplets had a strong odour, whereas female strobili were less fragrant. Probably attracted by the odour, nocturnal moths of Pyralidae and Geometridae visited the strobili between 18:00 and 21:00 h. We found nine pyralid and four geometrid species. Each moth landed on a strobilus and extended its proboscis to suck the droplets (*c*, *d* in the figure). Moths visiting male strobili sometimes probed the nectar secreted among male flowers and then their probosces touched the microsporangia. Moths fluttered slowly between strobili and the mean time spent on a strobili was 485 s (n=5, range 72–1,155). Moth visits per strobilus per night were more frequent on a male (1.74 ± 0.62, n=6) than a female strobilus (0.67±0.28, n=3). Each male and female strobilus was visited about 8.7 and 3.4 times on average respectively during the flowering period, which is about 5 days.

The pollen grains are inaperturate and sticky while a pollenkitt is absent. All the 18 moths collected on male strobili had pollen grains attached on probosces and antennae and, much less frequently, on tarsi of legs. Although there were no pollen grains attached on a moth collected on a female flower, the same species was collected on male strobili.

Most *Gnetum* species, even tall climbers, flower in the understory of rain forests where the humid habitat is least suitable for anemophily. *Gnetum* strobili, by emitting droplets as a nectar reward and odour as an attractant, attract nocturnal moths. *Gnetum* does not have typical moth flowers in a classical anthecological sense because petals are absent and the nectar reward is exposed. However, the nocturnal pattern of droplet secretion, the low sugar concentration of the droplet and fragrancy coincide with the moth-pollinated flower syndrome. High relative humidity and stillness of surrounding air in the evening favour this pollination system, which is also adopted in *Nepenthes* (Nepenthaceae) in the Pálaeotropics [10]. We believe that the moth attraction in *Gnetum* derived from unspecialized entomophily as observed in *Ephedra* and *Welwitschia* after the divergence of long-tongued lepidopterans which coevolved with deep angiosperm flowers in the late Cretaceous.

Makoto Kato
Biological Laboratory, Yoshida College, Kyoto University, Sakyo, Kyoto 606-01, Japan
Tamiji Inoue
Centre for Ecological Research, Kyoto University, Shimosakamoto, Otsu 520-01, Japan

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