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Studies on hitherto unknown fruits and seeds of some Rafflesiaceae, and a method to manually pollinate their flowers for research and conservation

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A b s t r a c t : The fruits and seeds of *Sapria himalayana* and *Rafflesia kerrii* are described for the first time. The fruits of the two genera clearly differ ontogenetically, in shape, size and colour. They are fissured berries: blackish, flattened barrel-shaped, 21-32 cm in circumference and 3.1-5 cm long in *S. himalayana*, but red-brown, truncated cone-like, 40-51 cm in circumference and 7.5-11.5 cm long in *Ra. kerrii*. The minuscule seeds are very similar, 0.6-0.65 mm by 0.29-0.33 mm in *S. himalayana*, a third larger in *Ra. kerrii*. A technique to manually pollinate *Sapria*, *Rafflesia* and *Rhizanthes* (Rafflesiaceae) was devised, using appropriately bent aluminium strips to reach the concealed sexual parts to acquire and deposit pollen, after excising parts of the flower in some of the species. The fruiting rate of *S. himalayana* was thereby increased to 78% from 8-12% found in naturally pollinated populations. The pollen, exuded as a thick suspension that solidifies on the back of pollinating blow flies *Lucilia porphyrina* (Diptera, Calliphoridae), is rapidly re-liquefied on coming into contact with the stigmatic fluid. Dry pollen remained viable for up to three weeks to produce mature fruits and seeds. Flowers are not apomictic but rather require pollination for seed set. Remarkably, the ovary and ovules of unpollinated flowers grew for 4-5 weeks after anthesis but were dead in 6-9 weeks. The development of the ovary, ovules, fruit and seeds is documented from anthesis until the natural splitting open of the mature fruit 5-6.5 months later. If not protected, all fruits were eaten by rodents, probably wild rats, the presumed seed dispersers. In experiments, rats consuming a whole fruit passed some 15,000 undamaged seeds in their fecal pellets. From this and other evidence, it is concluded that endozoochorous seed dispersal is more likely than exozoochorous dispersal in *Sapria*, *Rafflesia* and *Rhizanthes*. Thanks to the 6-10 fold increase in fruit set achieved by manual pollination, the method is an effective new tool for the conservation of endangered Rafflesiaceae.

K e y w o r d s : Calliphoridae, conservation, dispersal, fruit set, pollination, *Rafflesia*, *Rhizanthes*, *Sapria*, seed development, Thailand.

Introduction

Flowers of Rafflesiaceae are notoriously rare, but even more so is information on their fruits. The first fruit and seeds of a *Rafflesia* and a *Rhizanthes* became known to science some 20 and 80 years after their flowers were described, respectively (BROWN 1844, HEINRICHER 1905). The mature fruit and seeds of *Sapria* have remained unknown for nearly 160 years, until this report. Sketches and description of bud and flowers of *S.*

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himalayana GRIFFITH were made when they were discovered in 1836 and the fruit was preserved for subsequent study (GRIFFITH 1845). Unfortunately, the spirit material deteriorated to such an extent that GRIFFITH's description of the fruit published eight years after its discovery was only "... to the best of my recollection" (loc. cit.). Furthermore, fruit and seed evidently were not mature at the time they were found with the flowers (12 November, 1836; GRIFFITH 1847; no date mentioned in GRIFFITH 1845). Also, in his description and drawing, GRIFFITH (1845, Fig. 4; Table 35) mentions only ovules, not mature seeds, evidently salvaged from the spirit material. In the most comprehensive study of seeds of Rafflesiaceae and allied families to date, BOUMAN & MEIJER (1994) treated only the ovules of *S. himalayana*, because mature fruits and seeds had not yet been found.

There are three main reasons why fruits of Rafflesiaceae remained so long unknown. First, compared to the majestic, colourful or even disconcerting aspect of the flowers of the three genera, fruit and seeds could hardly be more anticlimactic. Fruits look like pieces of a fissured old tree trunk, lumps of soil, or charred woody remains (Figs. 1g-i, 2a-c), whereas the seeds are minuscule (Figs. 3a-f), less than 1 mm long, and brownish. Fruits are thus easily overlooked in the forest understory, and the seeds are nearly impossible to find once dispersed from the fruit. Second, the rate of fruit set is, at least in *S. himalayana*, low (8-12%, this study). Finally, fruits are readily consumed by frugivores.

In the three genera to which this family of holoparasites has been recently reduced (TAKHTAJAN 1997), there are 13-20 species of *Rafflesia* (MEIJER 1997, NAIS 2001, BARCELONA & FERNANDO 2002, LATIFF & WONG 2003), four of *Rhizanthes* and three of *Sapria* (BÄNZIGER & HANSEN 1997, 2000, BÄNZIGER et al. 2000). So far, fruits have been described for 4 species of *Rafflesia*, though they have been seen or photographed in some 10; seeds have been described in 6 species (Table 1, 2). The mature fruit and seed of *Ra. kerrii* MEIJER and *S. himalayana*, are described for the first time below.

However, the most important result of this study is the development of a method to manually pollinate flowers of Rafflesiaceae. The dramatic increase in fruit set thus achieved can be used to enhance the survival chances of these rare, vulnerable or endangered plants. The technique also made it possible to study – for the first time in Rafflesiaceae – the various developmental stages of fruit and seed as a function of time, besides other aspects of the reproductive biology, such as pollen viability over time, and the seed dispersal.

The early pioneer work on Rafflesiaceae has been reviewed by MEIJER (1997). Research stalled for several decades with the advent of World War I, until MEIJER (1958, 1984) reawakened interest in it. There followed much new information on the taxonomy (loc. cit.), floristics (e.g., HANSEN 1972, 1973), biology (e.g., BEAMAN & ADAM 1984, HIDAYATI et al. 2000, PATIÑO et al. 2000, NAIS 2001), ecology (e.g., BÄNZIGER 1991, 1995) and conservation of the plants (ISMAIL 1988, ELLIOTT 1990, NAIS & WILCOCK 1998). Yet, essential aspects of reproductive biology – including morphology and development of fruit, development and dispersal of seed, and, above all, the mechanism of host infection – have remained poorly known or objects of mere speculation. Only pollination syndromes have been studied to a certain extent (e.g., BEAMAN et al. 1988, BÄNZIGER 1991, 1996, 2001, BÄNZIGER & PAPE 2004). These are tantalizing scientific lacunae but also a serious impediment to conservation of the Rafflesiaceae.

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Table 1: The status of knowledge about fruit and seeds of *Rafflesia* species prior to this study.

Species	Details treated	Authors
R. arnoldii R. BR.	Structure of fruit, ovary, ovule, seed, development of ovule, figures	BROWN 1844
	Details on morphology and development of ovules and seeds, figures	SOLMS-LAUBACH 1874, 1898
	Size of fruit, figures	JUSTESEN 1922
	Saw many fruits (no description), hunter Saan saw squirrels eating fruit	MEIJER 1958
R. azlanii LATIFF & WONG	Photograph of ripe fruit	LATIFF & WONG 2003
R. gadutensis MEIJER	Structure of ovule, seed; micrograph	BOUMAN & MEIJER 1994
	Fruit needs 8 months to mature	MEIJER 1997
R. hasseltii SURINGAR	Description of fruit	ERNST & SCHMIDT 1913
R. keithii MEIJER	Structure of ovule and seed	BOUMAN & MEIJER 1994
	Photograph of whole and sectioned fruit, photograph of seeds	NAIS 2001
	Notes on fruit and seeds, photograph of squirrel eating fruit	EMMONS et al. 1991
R. kerrii MEIJER	Photograph of young fruit	PICHEANSUNTHORN et al. 2002
R. micropylora MEIJER	Structure of ovule, micrograph	BOUMAN & MEIJER 1994
R. patma BLUME	Description and figure of fruit	DE VRIESE 1853, 1854
	Description of fruit, seed, figure of seed	ERNST & SCHMID 1913
	Size of fruit and seeds	HIDAYATI et al. 2000
R. pricei MEIJER	Photograph of a gnawed fruit	NAIS 2001
	Photograph of a whole fruit	KULIP in NAIS 2001
R. rochussenii TEIJSM. & BINN.	Details of development of ovule, seed	SOLMS-LAUBACH 1898
	Description and figure of fruit	DE VRIESE 1853, 1854
	Description of fruit	ERNST & SCHMID 1913
	Morphological and anatomical details of seed, micrograph	BOUMAN & MEIJER 1994
R. tuan-mudae BECCARI	Photograph of young fruit	NAIS 2001

Materials and Methods

The main field study of *S. himalayana* was carried out on 21 clusters (a cluster is the buds and flowers parasitizing a single host) in an area in N Thailand not revealed here for conservation reasons. The habitat was hill evergreen forest at 1000-1450 m a.s.l. Study sites of *S. ram* BÄNZIGER & HANSEN, *Ra. kerrii* and *Rh. infanticida* BÄNZIGER & HANSEN were in W and S Thailand. The vulnerable or endangered status of many Rafflesiaceae due to rarity, susceptible parasitic life cycle, very high natural bud mortality, low

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reproductive capacity, habitat destruction, collecting for purported medicinal value, and ecotourism, has been mentioned by many authors (e.g., BEAMAN et al. 1988, BÄNZIGER 1988, 1991, NAIS 2001). Flowers are unisexual (except sometimes in *Rh. zippelii* (BLUME) SPACH, not present in the study area), possibly monoecious in *Sapria* and *Rhizanthes* (flowers of both sexes present in every cluster studied, though this may be due to a second infection by an individual of the other sex) but possibly dioecious in *Rafflesia* (both sexes rarely present in the same cluster)(BÄNZIGER 1995; this study). Pollen is presented as a droplet of "mush", i.e. a suspension of the consistency between mayonnaise and milk (loc. cit.). In the open air the matrix goes through a drying process in which the droplet clots and hardens with the pollen grains embedded (Figs. 4a, b); this process is reversible in the presence of water or stigmatic fluid. In normal early afternoon microclimatic conditions November-February (75-85% RH, 15-25°C), a fresh droplet placed on a microscope slide becomes covered with a dry film in 5-10 minutes; after 15-20 minutes the droplet has clotted but is still somewhat soft; after one hour it has hardened. In the generally more humid habitat of *Rafflesia* and *Rhizanthes* the process takes 2-3 times longer (loc. cit.). The amount of suspension present at an anther was assessed by measuring the droplet's dimensions under a stereomicroscope. The number of pollen grains per droplet was estimated from homogeneous smears of droplets on microscope slides; the grains were counted in microquadrats of a microscope grid and extrapolated. Natural pollination was studied by observing flowers (cf. BÄNZIGER 1991, 1996, BÄNZIGER & PAPE 2004) for 226 hours (*S. himalayana*) and 183 hours (*Ra. kerrii*), 1991-2002.

Table 2. The status of knowledge about fruit and seeds of *Rhizanthes*[a] species prior to this study.

Species	Details treated	Authors
Rh. deceptor BÄNZIGER & HANSEN[b]	Description of seed, micrograph	BOUMAN & MEIJER 1994
Rh. infanticida BÄNZIGER & HANSEN [c]	Photograph of unripe fruit	BÄNZIGER 1995
Rh. zippelii (BLUME) SPACH	Mention of ovule	SOLMS-LAUBACH 1874
	Description of nearly mature fruit and seed, figures	HEINRICHER 1905
	Mention of young fruit	ERNST & SCHMID 1913
Rhizanthes species[d]	Description of mature seed	BOUMAN & MEIJER 1994

[a]The genus has recently been revised resulting in major taxonomic changes (see BÄNZIGER & HANSEN 2000).

[b]As *Rh. zippelii* sensu MEIJER & VELDKAMP 1988; MEIJER 1997.

[c]As *Rh. zippelii* sensu MEIJER & VELDKAMP 1988; sensu BÄNZIGER 1995; as *Rh. lowii* sensu MEIJER 1997.

[d]As *Rh. lowii* sensu MEIJER 1997, actually an insufficiently known taxon.

In order to establish whether the flowers of *S. himalayana* are apomictic or not, pollinator (carrion flies) access was prevented. Large buds were enveloped in a green net (mesh gap less than 0.5 mm, net diam 30 cm, secured at the base of the bud) from before to at least one week beyond anthesis (flower dark, no foul smell, hence not attractive to polli-

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nators). The natural rate of fruit set of *S. himalayana* was assessed by comparing presence/absence of fruit production in untreated female flowers. The totality of female flowers was counted in all clusters, throughout the flowering season (September-April) of 2000-2003.

Experiments to manually pollinate *S. himalayana* were first attempted with Dr. S. Elliott in 1992 but were not successful. Beginning in 1996, I devised new methods, which finally met with success. Flowers of both sexes were protected from pollinators as described above. Pollen was obtained from fresh male flowers (not more than 4 days old) by first cutting off the diaphragm along its attachment to the tube with a small knife. Then the disk margin (about the distal 0.5 cm) was held fast between thumb and forefinger of both hands, on opposite sides of the disk. The disk was slowly wrenched off by both turning and pulling it up until the disk support tore, releasing the disk with its 20 anthers set in a circular row on the underside. For transportation the disk was forced into a film canister, base first and in such a way that the anthers did not touch the canister walls. In this way the pollen suspension did not coagulate for many hours.

Female flowers selected for manual pollination were not older than about 4 days and grew in different clusters than males from which pollen was taken. The diaphragm was removed as in the male. (Cutting off the very thin diaphragm has no apparent negative effect on the flower and prospective fruit, and at any rate seems to be less deleterious than cutting windows into the tube; the aesthetic damage to some specimens seems an affordable price to pay, if the survival of rafflesias can be improved.) Because the stigma, set on the underside of the disk, is not directly visible from above, a dentist's angled mirror was introduced between the disk and the tube and positioned in such a way as to reflect the image of a section of the stigmatic fascia. Normally, there was enough light filtering through the canopy for the stigma to be seen because the disk of female flowers is whitish and so somewhat translucent. If the site was too dark, a small electric light (3 Volt) was placed obliquely onto the flower in such a way that the light shined through the disk to reveal the stigma by transillumination.

For deposition of pollen onto the stigma, a strip of aluminium sheet was used, 0.5-1.0 cm wide, 3-4 cm long and 0.2-0.3 mm thick (i.e., sufficiently stiff to keep its shape but flexible enough for gentle manipulation). The strip was bent into an 'S'-like shape to facilitate its introduction into the female flower. The pollen from two to four anthers was tipped onto the distal end of a strip. Under inspection through the mirror, the strip was introduced into the space between the female disk and the tube until it was opposite the stigmatic fascia, when the pollen was smeared onto the stigma with a tangential, radial or diagonal motion. This was repeated with two or three strips at different sections of the stigmatic fascia. Thus the androecium of one male flower was sufficient for pollination of three female flowers with each receiving pollen of 6-8 anthers, the maximum amount seen on pollinators (see below for estimation of pollen load).

To study pollen longevity in the dry state, the procedure was the same but the aluminium strips tipped with fresh pollen were left to dry in the air. The amount of pollen used (1-10 anthers) and the date of treatment were inscribed on each strip. This was placed into a film canister, closed with brass mesh (mesh size 0.1-0.2 mm) to prevent the pollen from being eaten by various animals, and stored for the required length of time (1 day to 5 weeks) in ambient conditions out of direct sunlight at the study area, until use on subsequent days. During manual pollination, strips were introduced below the disk and

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brought into contact with the profuse stigmatic fluid, as described above. Dry pollen masses became re-liquefied in a few seconds (somewhat longer with very old pollen) and could be wiped off the aluminium strips. Treated and untreated female flowers were marked with a label and if its location in the forest was difficult to find, the distance and compass bearings relative to conspicuous landmarks were noted.

Pollen loads of *S. himalayana* on pollinators were measured as anther equivalents of pollen. The loads were compared to a series of different, known loads obtained in experiments. Known loads were obtained by applying pollen from one, two, four, six, eight, ten anthers to one each of six dead specimens of pollinators carrying no pollen. The best match between natural and experimental loads was used to estimate the amount of pollen carried by pollinators.

When developing fruits became attractive to rodents, at about 4 months age, they were covered with sturdy basins of hard plastic (diameter 30 cm, height 15 cm), amply perforated (2-5 mm diameter) for aeration. Before firmly staking the basins to the ground with 20 cm long rods, naphthalene balls were placed on top of the fruit and in a circle around the fruit as an additional measure to keep vertebrate and invertebrate frugivores at bay.

Manual pollination of the other species of *Sapria* was easier because the various passages – diaphragm aperture, spaces between disk-diaphragm and disk-tube – are wider than in *S. himalayana*. Generally, no excising of the diaphragm was needed. Manual pollination in *Rhizanthes* was easiest because it lacks the diaphragm and the anthers and stigma are readily visible and accessible to an aluminium strip. *Ra. kerrii* is so large that two hands easily go through the aperture, and a small electric torch and mirror can be placed onto the base to follow events. However, the anthers are too secluded to be seen even with the help of a mirror. Nevertheless, pollen can be obtained in two ways. One is on an aluminium strip 6-7 cm long, 1.5 cm wide at the base but only 0.3-0.5 cm wide at the tip, suitably recurved. The strip is introduced along one of the channels leading to the anther chamber, using the mirror and illumination for guidance. With experience, the position of the pollen at the anther can be estimated and a drop acquired. Alternatively, a pollen drop can be acquired on the tip of the researcher's finger which, if thin enough, can be advanced along a channel to an anther (some forcing may be necessary). Pollen can be readily transferred from the fingertip to an aluminium strip. Pollen deposition onto the huge stigmatic fascia is very easy because it is readily visible in the mirror.

The acceptability of the fruit of *S. himalayana* as food to presumed seed dispersers (i.e. various species of sylvatic rodents) and the ability of the seed to withstand mastication and digestion were tested with substitute rodents. A brown rat (*Rattus norvegicus* BERKENHOUT) trapped in a local market, laboratory white rats (same species) and, in a few trials, laboratory white mice (*Mus musculus* L.) were used. Four mature fruits 5 and 6 months old were given. Chunks of 1-2 cm^3 of *S. himalayana* fruit pulp with numerous seeds were offered with or without commercial guinea pig food and water to check food preference. The amount and condition of seeds in rat fecal pellets were analyzed by dissection of pellets with watchmakers' forceps under a stereomicroscope (10-40x magnification). Counts were carried out using pellets excreted 24 hours after the guinea pig food was discontinued.

Seed micrographs were made by scanning electron microscope by Dr. A. Blarer, Institute for Systematic Botany, University of Zürich, Switzerland. When required, the outer periclinal wall was removed by acid and ultrasound treatment.

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Results

Description of hitherto unknown fruits and seeds

Sapria himalayana (Figs. 1, 2a, b)

When fully developed, but before dehiscence, the fruit is a dark brown to black, flattened barrel-shaped berry of 21-32 cm circumference, 3.1-5.0 cm length, and 250-375 g weight. The fruits are laterally convex, the apex is a disk 6-8.5 cm in diameter, and the base is formed by the cupule 6-7 cm in diameter, that is attached to the host. Apically there may be more or less evident remnants of the radial ridges of the flower's tube base and fragments of the tube as a circular, irregular ridge along the margin of the fruit's apex. There are cracks all over except the cupule. The lateral walls are dissected with horizontal cracks up to 7 cm long, 0.5 cm wide and 0.5 cm deep, and by vertical cracks that are generally smaller. The apex has large radial cracks up to 3 cm long, 1 cm deep and 1 cm wide, with or without additional smaller radial cracks, and often large, more or less circular cracks around the remnants of the attachment of the original flower's disk. Occasionally, the shrunken, pitch black perigone tube, lobes, bracts, and disk, whole or in parts, may remain attached to the fruit.

The pericarp is 6-13 mm thick. It consists of a dark exocarp 2-8 mm thick, a reddish mesocarp 2-7 mm thick, and a yellowish endocarp 1-3 mm thick, below which is the white pulp of the fruit filling a space of 7-7.5 cm in diameter and 3-3.5 cm in depth. The pulp is intersected by many septa bearing large numbers of tiny seeds. When dehisced (Fig. 2a), the fruit somewhat resembles a pan, 9.2-13 cm in diameter, in which the fruit pulp and seeds are exposed. The gaps between the split portions of the fruit are 2-8 cm wide.

The ovules are 0.27-0.35 mm long and 0.09-0.13 mm wide at anthesis. The mature seeds (Figs. 3c-f) measure 0.6-0.65 mm long and 0.29-0.33 mm wide (Table 3). They are "J" shaped with a yellowish, recurved, faintly pitted raphal portion, and an ellipsoidal, micropylar portion which is enclosed in a brown, very hard, irregularly hexagonal, reticulation of the seed coat with pale yellow pits.

Sapria ram

From the remains of two fruits (content eaten and part of the walls broken by rodents), their circumference was ca. 17.5 cm and length 3 cm. Many cracks were present in the wall. No seeds remained.

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Table 3: Developmental stages of ovules and seeds of 15 developing fruits of *Sapria himalayana* harvested at different ages from anthesis to fruit maturity. Flowers were hand-pollinated except where otherwise indicated. Size ranges in mm.

Age	Total length of ovule/seed	Raphal portion		Micropylar portion		Ovule/seed exotegmen texture	
		width	colour	width	colour	raphal portion	micropylar portion
Anthesis	0.27-0.35	0.09-0.10	all white	0.1-0.13	white	amorphous	amorphous
20 days	0.35-0.38	0.10	all white	0.10-0.13	white	amorphous	amorphous
37 days	0.42	0.10	basal ½-⅔pink-violet, rest white	0.10-0.13	white	amorphous	amorphous
46 days	0.3.9-0.42	0.10	basal ½-⅔pink-violet, rest white	0.13-0.14	white	amorphous	amorphous
60 days	0.48	0.13-016	basal ½-⅔pink-violet, rest white	0.21	white	pitting starts to show	amorphous
78 days	0.52-0.55	0.16	all white	0.27-0.29	white	pitting clearer	amorphous
84 days	0.57-0.60	0.18	all white	0.29-0.31	white	pitting clear	pitting starts to show
99 days	0.57-0.62	0.18-0.19	all white	0.29-0.31	white	pitting clear	pitting clear
118 days	0.59-0.62	0.18-0.21	all white	0.31	white	pitting clear	pitting clear
126 days	0.62-0.65	0.23	all white	0.31	white	pitting clear	starts to harden, pitting clear
143 days[a, b] ± 3 days	0. 62-0.65	0.16-0.21	pale yellow	0.29-0.31	brownish	pitting clear	hardening, pitting clear
151 days[c]	0.60-0.65	0.18-0.21	pale yellow	0.29-0.31	brown	pitting clear	hard, pitting clear
164 days[c]	0.65	0.21	pale yellow	0.31	brown	pitting clear	hard, pitting clear
180 days[b]	0.6-0.65	0.23	pale yellow	0.3-0.33	brown	pitting clear	hard, pitting clear
193 days[a, b] ± 3 days	0.61-0.65	0.18-0.23	pale yellow	0.31-0.32	brown	pitting clear	hard, pitting clear

[a]Was naturally pollinated, but the pollinator was not seen; the age at harvest time is counted starting from the fourth day of anthesis ± 3 days.
[b]Fruit split open naturally.
[c]Partly eaten by rodents.

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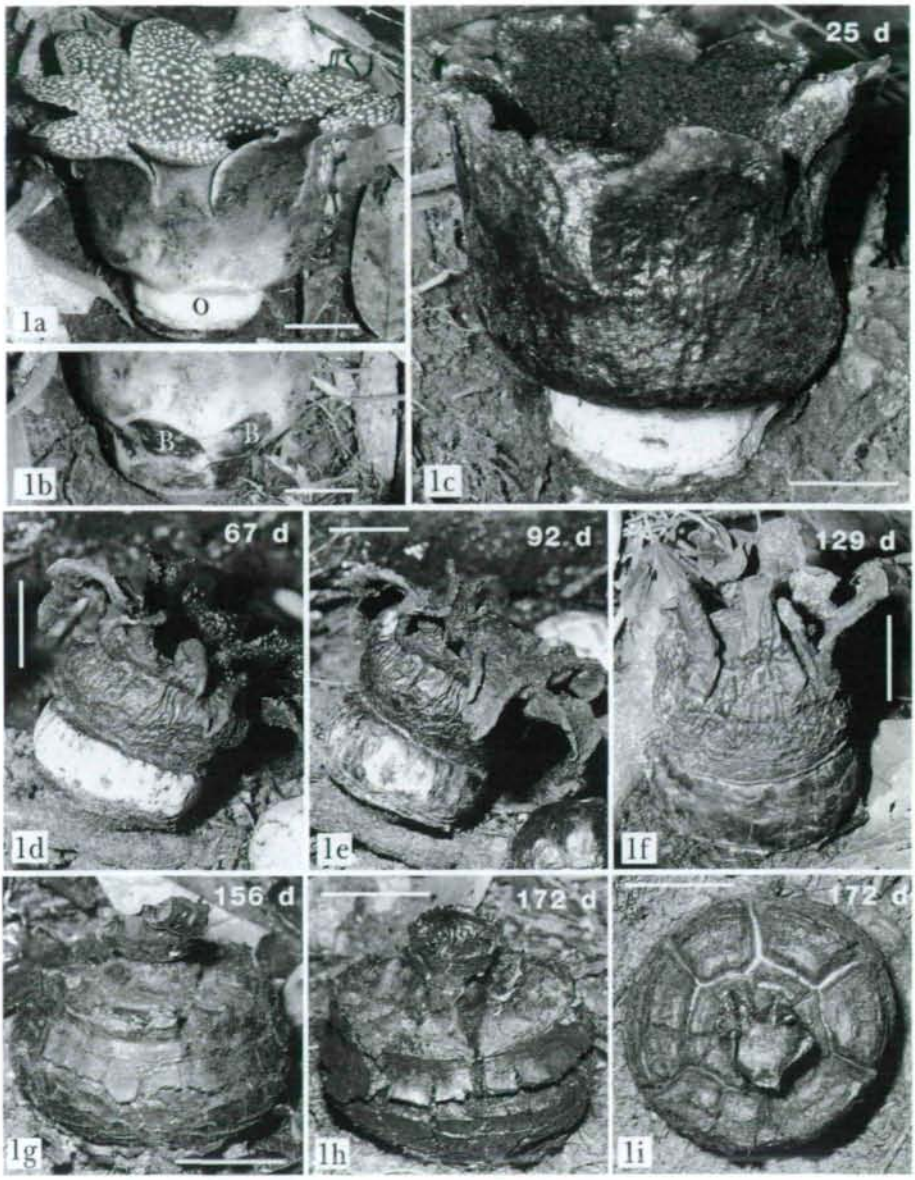

Fig. 1a-i: Flower and fruit development of *Sapria himalayana*. **1a-b** – Female flower at anthesis, after and before removal of the bracts covering the ovary. O= ovary, B=bracts. **1c** – Scenescent flower parts and post-anthesis ovary wall, 25 days old (note the white wall and black floral tube and lobes); **1d** – Same, 67 days old (note the enlarged wall and shrunken floral parts); **1e** – Same, 92 days old (note the darkening wall); **1f** – Same, 129 days old (note the small cracks in the wall); **1g** – More or less ripe fruit, 156 days old (note more conspicuous cracks); **1h-i** – Ripe fruit, 172 days old (laterally and dorsally seen, note the deep and wide cracks). d=days. Bar length=3cm.

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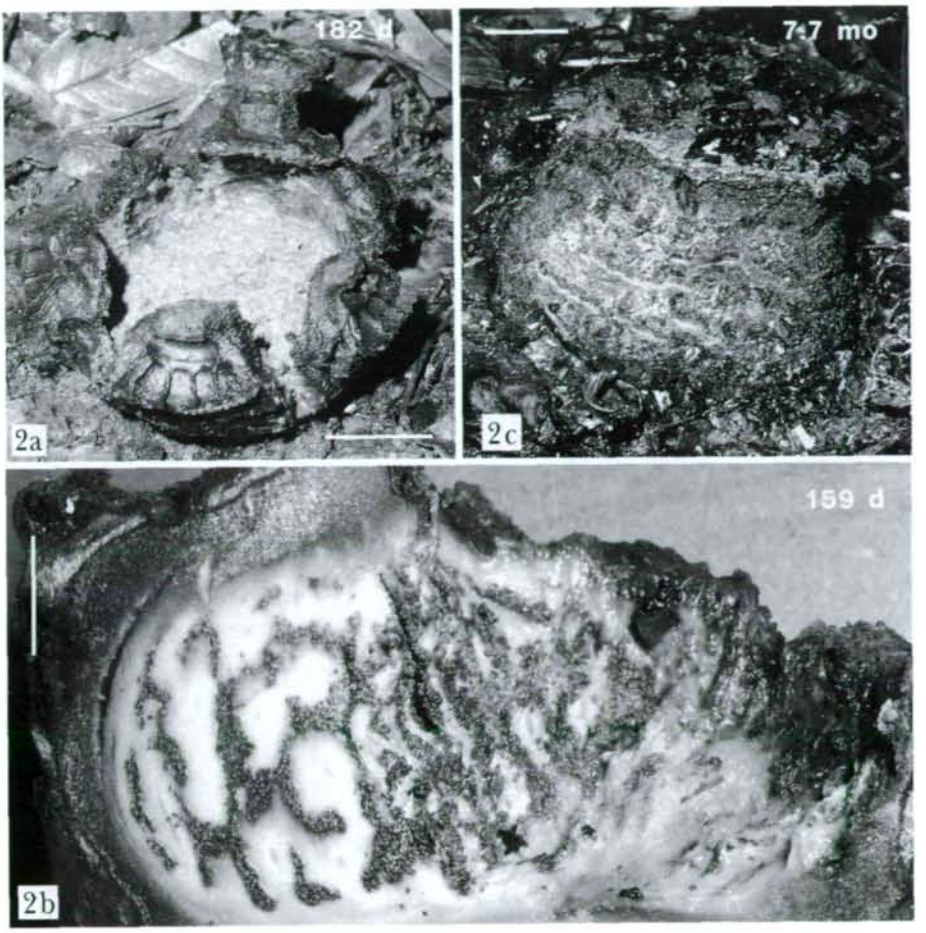

Fig. 2a-b: Fruit of *Sapria himalayana*. **2a** – Overripe fruit, 182 days old, fully opened naturally (note the water-soaked, pale, decomposing fruit pulp). Bar length=3 cm. **2b** – Cross section of a fruit 159 days old, showing the seed arrangement and the white pulp; part of the upper right portion has been eaten by a rodent. d=days. Bar length=1 cm. **Fig. 2c:** Fruit of *Rafflesia kerrii*. – Ripe fruit, 7.7 months old. mo=months. Bar length=3 cm.

Rafflesia kerrii (Fig. 2c, Table 4)

Eight mature fruits (aged 6.5-7.7 months) were measured *in situ*, but only one was collected for studying the seeds. The shape of the fruit may be best described as similar to an overturned flower pot. The main body is a red-brown cone with convex walls, 7.5-11.5 cm high, 40-51.2 cm in circumference at the base and 25.6-34 cm in circumference at the circular constriction below the top. This is capped by a more or less evident disk, 19-37 cm in circumference. There are cracks all over, but in the specimens seen they were rather less obvious than in *S. himalayana* and some of the *Rafflesia* species that have been illustrated (e.g. BROWN 1844, NAIS 2001).

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The ovules (taken from a moribund flower) are 0.62-0.73 mm long and 0.29-0.31 mm wide. The seeds (Figs. 3a, b) are 0.86-0.99 mm long and 0.39-0.44 mm wide. They have essentially the same shape and colour as in *S. himalayana* but the reticulation of the seed coat is slightly wider-meshed in *Ra. kerrii*.

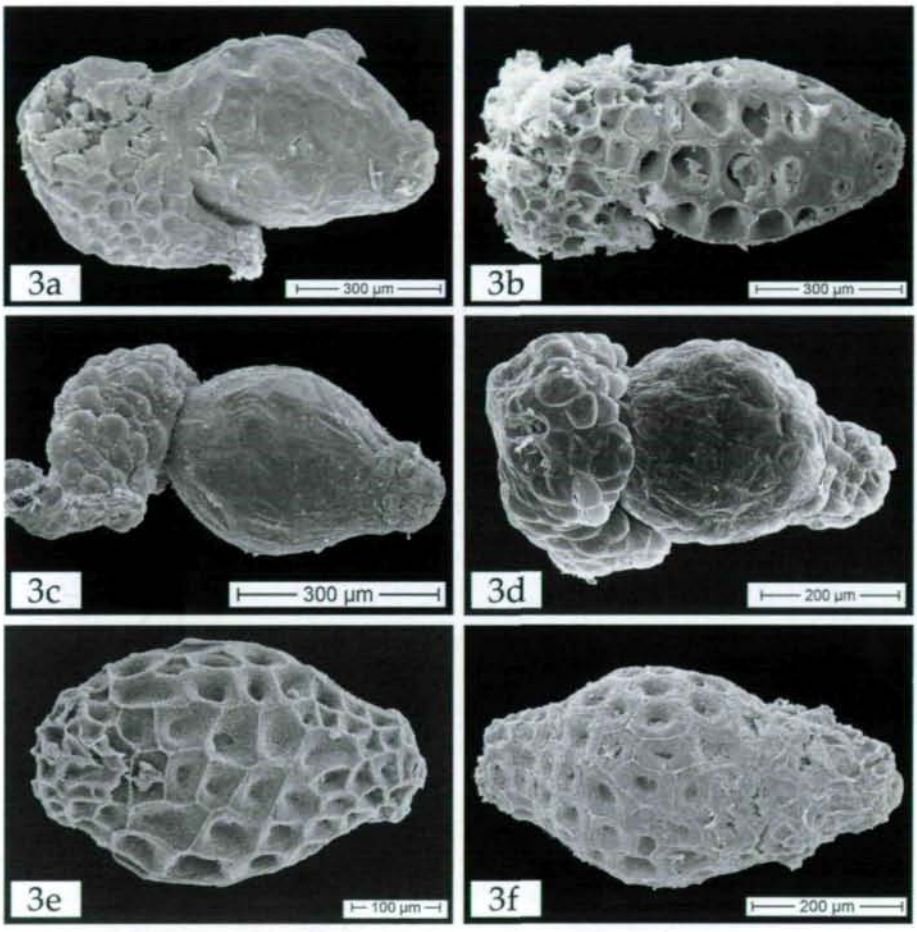

Fig. 3a-b: Seeds of *Rafflesia kerrii*. **3a** – Before removal of the periclinal wall. **3b** – After its removal, both from naturally pollinated flower. **Fig. 3c-f:** Seeds of *Sapria himalayana*. **3c** – From naturally pollinated flower, before removing the periclinal wall. **3d** – From flower pollinated manually with 24 days old pollen, before removing the periclinal wall. **3e** – Same but after removing the periclinal wall. **3f** – Seed recovered from rat excrement, most of the periclinal wall naturally removed by digestion. Micrographs by Dr. A. Blarer.

Breeding system of *Sapria himalayana*

None of the 35 flowers from which pollinators were excluded produced mature fruits or seeds, although they survived and even grew for a few weeks after anthesis (see below). All were clearly senescent after 6-9 weeks. This peculiar development of the ovary and ovules is described in more detail below.

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Table 4: Dimensions of fruits of *Rafflesia kerrii*, with date and age at last sighting. Sizes in cm.

Code	Date age (months)	Circumference at base	Circumference at constriction	Circumference at top (disk)	Height
HY2	29.11.91 7 ½	45.5	34	37	10
HY13a	30.11.91 about 7	41	----------	27.3	7.5
HY13b	30.11.91 about 7	40	----------	30.1	7.5
HY16.1	16.9.92 less than 7	50.4	27.3	30.4	11.5
HY13.4[a]	8.11.92 about 6 ½	42	33	34.5	10
LLBa	10.8.93 unknown	51	----------	19	9.5
LLBb[b]	14.8.93 unknown	51.2	25.6	32.1	9
PY	10.4.01 unknown	47	33	35	8

[a]Was a bud of 41 and 55 cm circumference on 26.2.92 and 4.4.92, respectively, and opened before mid April.
[b]This is the only fruit collected, the seeds were mature: 0.87-0.94 mm long, dark brown and hard.

Development of the post-anthesis, non-pollinated flower, ovary and ovules of *Sapria himalayana*

Essentially, the changes in size and colour during the first 5-7 weeks were the same as in the pollinated flower - withering and blackening of the superior parts of the flower but expanding of the consistently pale lateral wall of the ovary. Subsequently, however, the ovary wall stopped expanding and slowly discoloured to yellowish and then brownish (moribund stage). When 6-9 weeks old, the wall turned black and shrank. Internally, the pulp was found to be decomposing, turning more or less yellowish to brownish from its original white colour. At this stage the ovary was considered dead.

The ovule length, before decomposition, was 0.37-0.48 mm. This is clearly longer than the ovules at anthesis (0.30-0.35 mm, Table 3), but comparable to the ovules of pollinated flowers of the same age. Similar to the ovules of pollinated flowers, non-fertilized ovules assumed a pinkish violet colour at the basal raphal part; subsequently, as they started dying, they turned yellowish to brownish when 40-60 days old.

Development of the fruit and seed in the pollinated flower of *Sapria himalayana* (Figs. 1, 2a, b; Table 3)

At anthesis, the flower was red with yellow-dotted perigones internally (Fig. 4a), but externally it was white on about the basal half and plain red on the distal portion. Towards the end of anthesis, which in females lasted 6-7 days, the coloration turned increasingly brownish and then blackish at some 7-15 days (high temperature and low or

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high humidity seemed to accelerate deterioration). In 30-50 days, the perigone lobes, tube, disk and bracts shrank to about 2/3 of their blooming size (flower diameter 12.3-22.2 cm) or slowly rotted. (Unlike *Rafflesia*, which rots very quickly to a black slimy mess, decomposition in *Sapria* is, as in *Rhizanthes*, very slow.) Black, skeletonized remnants may still be extant one year later. Below the bracts, the lateral wall of the post-anthesis ovary remained white for some 60 days and expanded mainly in circumference, while the top wall blackened along with the other parts of the flower. The lateral wall, initially 16.3-20.4 cm in circumference compared to the floral tube circumference of 28-34 cm, enlarged to reach the same circumference as the shrinking floral tube in 30 to 55 days, when both were 18-21 cm in circumference. After 60-80 days, the lateral wall slowly changed colour to yellowish with brownish dots or flecks. At the age of about 80 days, expansion in length started to become evident and the first small cracks appeared, although in some large fruits these became evident only at about 110 days old. When 110-130 days old, the lateral wall generally had become uniformly black brown, although in some large fruits this occurred when 160 days old. There were now numerous very small cracks, but also several very large and deep ones positioned horizontally and vertically on the lateral wall, radially and more or less circularly on the apical surface. When 150-195 days old, the fruit reached maximum size, i.e. 21-32 cm in circumference and 3.5-5 cm length, after which it split open. Splitting occurred slowly along some of the cracks, which were as deep as the fruit's wall, now a rind 0.2-1 cm thick.

It was interesting to note that towards the latter part of the dry period from January to March there was often a slowdown of the expansion, or even a slight reduction, in the circumference of the fruit; this was reversed when the rains started again.

At anthesis, the ovules were 0.30-0.35 mm long and 0.10-0.13 mm wide. They were completely white until 20-30 days old, when they started to become pinkish violet on the basal half of the raphal part, but white elsewhere. When about 50 days old, the micropylar portion started widening. After the age of about 60 days, they were again all white and the pitting of the raphal portion began to be visible. In the micropylar portion the pitting occurred when some 80 days old. The seeds stopped growing when 0.62-0.65 mm long and 0.29-0.33 mm wide at an age of about 120 days. This was when the exotegmen started hardening (tested by squeezing it with forceps). When about 150 days old, the micropylar portion became brown and the raphal portion pale yellowish.

The pulp of the fruit was white from anthesis until the fruit split open, had a firm consistency (comparable to a peach) and the juice was acidic in taste and smell. It was not oily, unlike the pulp of *Ra. keithii* MEIJER (EMMONS et al. 1991), because it appeared to readily mix with rain water (see below). Some *S. himalayana* fruits had an odor reminiscent of santal fruit (*Sandoricum koetjape* (BURM. f.) MERR., Meliaceae), others smelled more like yogurt. If rain soaked a split-open fruit, the seeds became bathed in a fruit pulp of mushy consistency, yellow colour and, after some time, fecal-like smell. Adult beetles of two species of coprophagous *Odontophagus* (Scarabaeidae) were found wallowing in the pulp. Such foul odor is indicative of decomposition of proteins, probably an important constituent of the pulp.

From the above it can be concluded that the seeds need at least 5 months to mature. Fruits are mature when 5-6.5 months old, at which time they split open, most commonly in June-mid August.

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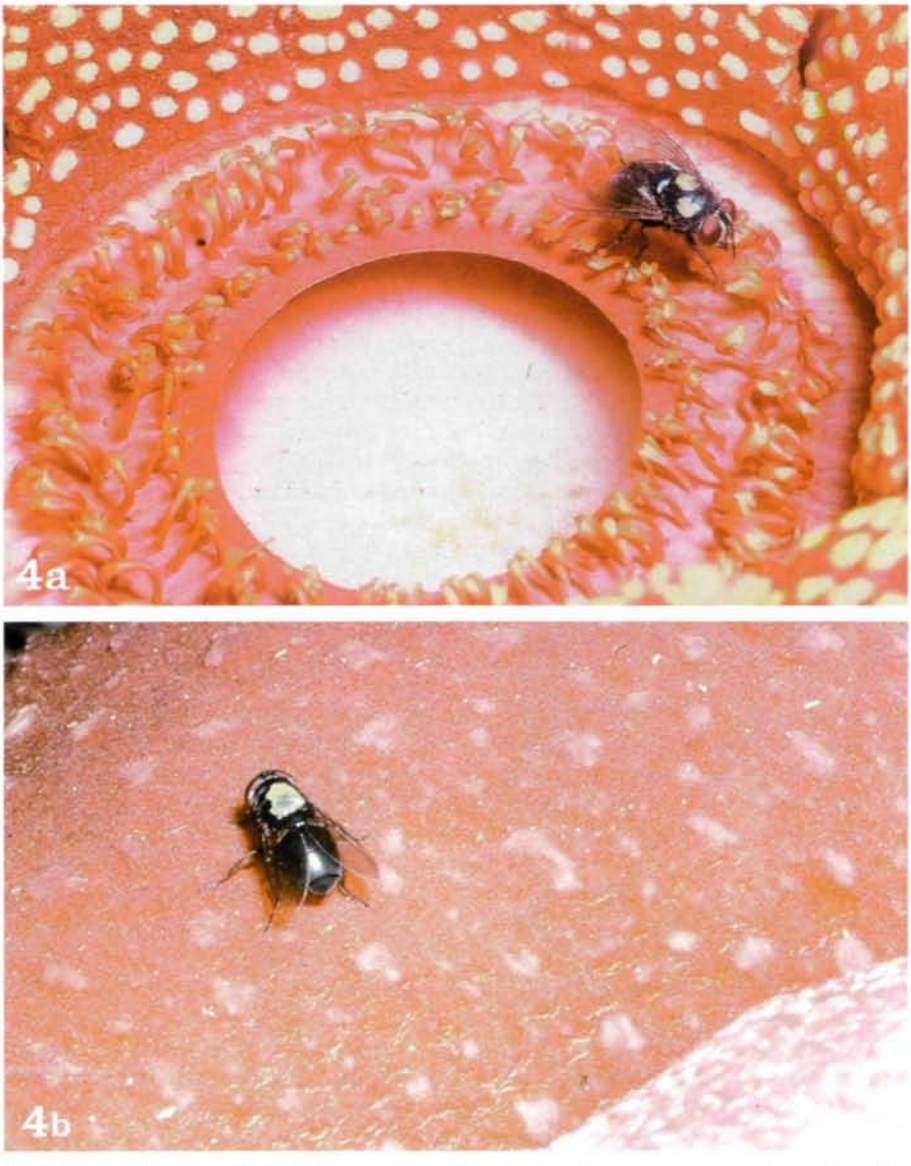

Fig. 4a-b: Pollinators of Rafflesiaceae. **4a** – Female *Lucilia porphyrina* crawling along the diaphragm of a female flower of *Sapria himalayana*; note the blow fly carrying two separate, diagonally set, dry pollen drops acquired during a previous visit of a male flower. **4b** – Female *Chrysomya villeneuvi* resting on the perigone lobe of *Rafflesia kerrii*; note the blow fly carrying a large, dry pollen smear acquired during a previous visit of *Ra. kerrii*. Both flies are about 1 cm long.

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Pollinators, amount of pollen acquired, and rate of fruit set under natural conditions

Pollinators of *S. himalayana* are female (rarely male) blow flies *Lucilia porphyrina* (WALKER) (Diptera, Calliphoridae)(Fig. 4a): 31 females and 4 males were caught carrying a smear, and many more were seen but not caught while entering or leaving the flower. Other fly species were only exceptionally involved. Visits tended to be sporadic, generally only one fly every 0.5-4 hours. Pollen on the back of flies flying in from elsewhere was mostly dry (Fig. 4a). The amount of pollen acquired by the pollinators examined varied from less than the amount normally presented at one anther, to an equivalent of about 6-8 anthers. Pollen from about 3-4 anthers was the most frequently seen. A pollinator generally acquires pollen more or less directly from several anthers during one visit. In several cases, individuals of *L. porphyrina* acquired a fresh smear of pollen on top of a dry one obtained from another flower. Hence pollen acquisition from flowers of *S. himalayana* is quite different from *Ra. kerrii* flowers. In *Ra. kerrii*, pollen is obtained from only one anther at a time because in *Rafflesia* each anther is set separately in its own chamber at the end of a channel (cf. Figs. 21-24 in BÄNZIGER 1991). On the other hand, the amount of pollen present at an anther of *Ra. kerrii* is some 15 times more than in *S. himalayana*, viz. over 30 mm^3 and 2 mm^3, respectively – not surprising in a flower with a diameter about four times that of *S. himalayana*. Pollen grains per anther number 1.5-2.4x10^6 and 1.3-1.8x10^5 in *Ra. kerrii* and *S. himalayana*, respectively. Thus, in *Ra. kerrii* the pollen suspension at an anther evidently has a slightly lower concentration of grains than in *S. himalayana*.

Pollinators of *Ra. kerrii* were female blow flies *Chrysomya villeneuvi* PATTON (Fig. 4b), *C. defixa* (WALKER), *C. chani* KURAHASHI, *C. pinguis* (WALKER), *C. rufifacies* MACQUART, of which 31, 20, 4, 4, and 3 specimens carrying pollen were collected, respectively (and even more were seen but not collected). Two further blow fly visitors, the rather small *C. nigripes* AUBERTIN and very large *Hypopygiopsis tumrasvini* KURAHASHI, do not fit properly the channel leading to the anther chamber and only very exceptionally can acquire a smear (only one specimen each caught with a smear). Flesh flies (several species of *Sarcophaga* (Diptera, Sarcophagidae)) occasionally visited the flowers but they are not pollinators, as is the case with many other insect visitors. All seven *Chrysomya* species carried less than the amount of pollen present at one anther, but the load sizes were generally comparable to those acquired by *L. porphyrina* from *S. himalayana*.

During every study year, three to ten flowers of *S. himalayana* were found to naturally develop a fruit to maturity in the study area (Table 5). This corresponds to 8-12% of the 32-82 unprotected female flowers surveyed each year 1998-2003.

From preliminary findings, it is clear that in *Ra. kerrii* fruit set is much higher than in *S. himalayana*, possibly 20-30%. This agrees well with the far more numerous pollinators seen visiting *Ra. kerrii*, where often several flies were observed to visit simultaneously throughout much of the day, with occasional peaks of some 20.

Frugivores or seed dispersers?

When fruits of *S. himalayana* were nearly or completely mature, but sometimes when only 2-4 months old, a wide hole was found to be gnawed into the wall, part of the walls were broken and the pulp, wholly or in part, was eaten with the seeds (Fig. 2b). Teeth marks – two parallel incisor lines – were often discernible on the wall and in the pulp,

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unmistakably those of medium-sized rodents. Similar observations were also made with two fruits of *S. ram*, 4.5 months old. Most probably, the marks were from forest rats, or perhaps less likely from squirrels (cf. Discussion). No fruit survived rodent attack to reach the stage when the fruit splits open, unless protected (cf. Methods). However, whereas a certain amount of immature fruit is destroyed by rodents, they also appear to play a vital role as seed dispersers, as evidenced below.

Table 5. Fruit set of *Sapria himalayana* under natural conditions, 1998-2003.

Flowering season	Number of non-enveloped female flowers surveyed	Number of flowers which produced mature fruit and seed	Number of flowers which did not produce fruit
1998-1999[a]	---	4	---
1999-2000[a]	---	3	---
2000-2001	37	3 (8.1%)	34 (91.9%)
2001-2002	32	3 (9.4%)	29 (90.6%)
2002-2003	82	10 (12.2%)	72 (87.8%)

[a]The number of non-enveloped flowers was not surveyed, only the fruits.
The much higher number of females investigated in 2002-2003 is probably due to a combination of an unusually profuse and very long flowering of *S. himalayana* following unprecedented rains which obliterated the dry season.

The efficiency of manual pollination

As shown in Table 6, the success rate of manual pollination of *S. himalayana* in experiments was high: 32 (78%) of the 41 manually pollinated flowers developed mature fruit and seed. This is a six- to ten-fold increase compared to 8-12% fruit set found in nature. The pollen amount used, six to eight anthers, is about the maximum seen naturally acquired by pollinators. However, it can be expected that half the amount would have yielded similar results, especially considering the results of Table 7.

No experiments were made to distinguish between the efficiency of fertilization of pollen in the fluid against the dry state. This is because, in the wild, the probability of fluid pollen being smeared by pollinators on the stigma is very low compared to dry pollen (Fig. 4a-b).

Table 6: The success rate of manual pollination as evidenced from fruit set of *S.himalayana*.

Number of trials	Mature fruit and seed developed	Fruit set not successful
41	32 (78 %)	9 (22 %)

Fruit set in relation to the amount and age of pollen used

Because of the unpredictability of anthesis and limited number of flowers available, it was not possible to make more systematic and numerous trials than the 41 shown in Table 7. Pollen amounts from 4 and 8 anthers (the most frequent and the maximum amount of pollen carried by pollinators, respectively), retained sufficient numbers of pollen grains viable for up to 3 weeks for the development of mature fruit and seed.

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Larger quantities of still older pollen were viable to some degree, but this would require that several pollinators deposited maximum amounts of pollen on the same flower, probably a rare event in *S. himalayana* (unlike *Ra. kerrii*). The smallest amount tried, from a single anther, was also viable, even when dried for three days.

Table 7: Fruit set in relation to the amount and age of pollen manually deposited onto the stigma.

No. of anthers used	No. of trials	Age of pollen in each trial (time unit: days, except where otherwise stated: h=hours)	Fruit set in each trial (fruit developed: +; fruit not developed: -)
1	3	1.5 h, 1.5 h, 3	+, -, +
2	8	3, 7, 7, 7, 7, 13, 16, 20	-, +, +, +, -, -, -, +
4	6	12, 15, 17, 17, 20, 21	+, +, -, -, +, +
8	11	4, 5, 5, 5, 5, 7, 12, 12, 12, 17, 24	+, +, +, +, +, -, +, +, +, -, +
10	5	5, 9, 11, 18, 25	+, +, -, +, +
16	2	11, 18	+, +
20	5	20, 24, 28, 30, 32	+, +, -, -, -
30	1	30	+

Feeding experiments with rodents

Feeding experiments show that laboratory rats and mice prefer standard rat chow over the fruit of *S. himalayana*, probably due to habituation. However, the fruit was accepted when no rat food was given. The brown rat, on the other hand, preferred the fruit over rat chow.

Ten typically-sized rat fecal pellets analyzed contained 105 to 686 seeds (most pellets had about 350-400 seeds). Some 10% of the seeds had a more or less large part of the exotegmen broken, evidently due to mastication. In about 90% of the seeds, the exotegmen was intact, these were probably swallowed intact without being subjected to grinding by teeth. The seeds also withstood digestion, because their walls remained hard (tested by squeezing with forceps). Unexpectedly, in the white mouse, despite its much smaller size and expected more thorough chewing, the amount of broken seeds was still relatively low, viz. in average about 15% of the 27-70 seeds (average 37) found per pellet.

Interestingly, as evidenced by SEM, the seeds' outer periclinal wall, which in many plant species has to be removed (e.g. by digestion) before germination can start, was more or less completely removed from excreted seeds (Fig. 3f). (When viewed in the stereomicroscope (50x), the periclinal wall is not evident: the seeds' reticulate coat and pitting is visible through the transparent, very thin periclinal wall. This may lead to the mistaken assumption that the periclinal wall is absent in seeds taken directly from fresh fruit).

The number of pellets produced from the ingestion of a whole, typically-sized fruit was about 50 in the rat. With 350-400 seeds per pellet, the total amount of undamaged seeds, which passed the alimentary tract of the rat eating one whole fruit, is extrapolated at more than 15,000.

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Manual pollination of species other than *Sapria himalayana*

Both of two trials made with manual pollination of *S. ram* yielded fruits but they were found to be eaten by rodents 4.5 months later, before I could study them. Pollen acquisition in *Ra. kerrii* was somewhat more difficult but successful. Unfortunately, because of the lack of females during the time of the study, it was not possible to try pollen deposition, though it is easily accomplished (cf. Methods). In *Rafflesia* species with a narrow aperture (e.g. *Ra. micropylora* MEIJER and *Ra. cantleyi* SOLMS-LAUBACH), the only manipulation needed will be cutting away part of the diaphragm. In *Rh. infanticida*, pollen acquisition was the easiest of all but no female was in anthesis during the study. It is evident that pollen deposition is as easy as its acquisition. Yet, one difficulty in *Rh. infanticida* is that, due to its generally high attractiveness to pollinators, its pollen is rapidly depleted, often during the morning of the first opening day. Hence, to obtain pollen for hand pollination work, the growth of the buds must be monitored carefully for prediction of time of opening (see BÄNZIGER 1995). Also, the stigma is wet for only one or at most two days, instead of for 5-7 days, as in the other genera (BÄNZIGER 1995, 1996).

Discussion

This study has established that the reproduction of *S. himalayana* is not apomictic but dependent on pollinators. None of the 35 females protected from pollination developed fruit and seed. Outcrossing experiments have been highly successful. However, whether xenogamy is obligatory or not in *S. himalayana* has not been possible to examine experimentally, for it is not known whether one or more individuals infect the same host. A relatively large number of trials was made to provide solid evidence for the lack of apomixis and for the unexpected finding that for 5-7 weeks after anthesis, unpollinated flowers of *S. himalayana* continued developing like pollinated flowers. The present study shows that the ovules evidently are not mature at anthesis, but why they do not senesce immediately after anthesis when pollination has failed, as in "normal" flowers, is not clear. Is it due to the parasitic nature of the Rafflesiaceae, where the energy requirements are met by the host, leaving no incentive to economize? Is *S. himalayana* on the way to evolve apomixis due to low pollinator service? The flower would have an ample temporal window in which selection could act.

It is interesting to note that by a different approach, SOLMS-LAUBACH (1898) and ERNST & SCHMID (1913) also concluded that ovules are immature at anthesis in *Rafflesia* and *Rhizanthes*. Cytological and embryological analysis of *Ra. arnoldii* R. BROWN, *Ra. hasseltii* SURINGAR, *Ra. patma* BLUME, *Ra. rochussenii* TEIJSM. & BINN. and *Rh. zippelii* showed that ovules were not yet fully differentiated at floral anthesis. However, these authors were working with incidentally collected buds, flowers and fruits, without knowledge of age and without experimental material for comparison. They explained the immature ovular state by comparing Rafflesiaceae with Orchidaceae, where the primitive developmental state of the ovules at anthesis requires pollination to activate further development. However, from the present findings, a mechanism other than pollination must be involved, because the ovules continued to grow after anthesis in unpollinated flowers. Whatever this mechanism is, fertilization seems necessary for development of mature seeds.

Post-anthesis development in unpollinated flowers of *Sapria* has some bearing on the controversy over whether *Rafflesia* is apomictic or not (reviewed by NAIS 2001). In some

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cases initial development of the ovary may have been misinterpreted as evidence that a particular unpollinated flower of *Rafflesia* is apomictic. On the other hand, it may be that in *Rafflesia* there are apomictic as well as sexual species. For example, flowers of *Ra. kerrii* are very frequently visited by pollinators (BÄNZIGER 1991, this study) whereas those of *Ra. cantleyi* are not (pers. observ.), so that absence or presence of apomixis in the two species, respectively, would be understandable as reproductive strategies. Unfortunately, I could not yet test the breeding system of *Ra. kerrii*. The question of the reproductive mode among species of *Rafflesia* is of fundamental importance, if their survival chances are to be improved.

Ontogenetically, the fruit of *S. himalayana* is clearly different from that of *Ra. kerrii*. In *S. himalayana* the lateral wall of the fruit is derived from the basal-most part of the perigone, viz. the part which is between the basal portion of the tube and the bract insertion (cf. Fig. 3 in BÄNZIGER et al. 2000). The apex of the fruit is derived from the base of the tube and column, and the bottom of the fruit is formed by the cupule. In *Ra. kerrii* the lateral wall is derived from the column and the apex from the disk. Only the bottom of the fruit is, as in *Sapria*, formed by the cupule. This has also been noted by ERNST & SCHMID (1913) for *Ra. hasseltii*, *Ra. patma* and *Ra. rochussenii*. Thus it is not surprising that the fruit of *Sapria* and *Rafflesia* are of different shape. Other differences are the radial and circular cracks versus irregular cracks on the top of the fruit, the blackish versus the red-brown colour, and the smaller versus larger fruit, respectively, in *Sapria* and *Rafflesia*.

The peculiar features of the pollen suspension of *Ra. kerrii*, *Rh. infanticida* (then as *Rh. zippelii*) and *S. himalayana* to (i) clot, (ii) re-liquefy on stigmas and (iii) retain germinability over a period of 10-20 days has been previously pointed out (BÄNZIGER 1995). Manual pollination now demonstrates that it also retains its ability to trigger the development of mature fruit and seed. Pollen from one, four and eight anthers retained enough active pollen grains for at least 3, 21 and 24 days (Table 7). The amount of pollen involved evidently plays a role, because the larger the pollen load, the higher will be the probability that it contains viable pollen grains over a longer time period. However, the amount of pollen may have an additional effect on pollen longevity. This is due to the faculty of the suspension to solidify and the fact that in a fruit like *S. himalayana*, producing tens of thousands of seeds, large amounts of pollen are required for fertilization (there are up to 1.8×10^5 and 2.4×10^6 pollen grains per anther in *S. himalayana* and *Ra. kerrii*, respectively; with 26-36 anthers, *Ra. kerrii* is among the flowers with the highest counts of pollen grains, close to 100 million). In massive loads of pollen – of up to 8 anthers on *L. porphyrina* – the microclimatic regime at the surface and the center of the load can be expected to differ, especially once the outer layers have solidified. These may act like a "shell" reducing further desiccation, insolation, collapsing and crushing of pollen grains, and may well also protect against infection. Dry pollen loads (Fig. 4a-b) remain attached to the fly life-long; except for re-liquefaction, the load cannot be removed by the fly because it would have to tear off also its own bristles firmly cemented in the hardened suspension. Long-term pollen viability not only increases the chances that flowers blooming weeks and considerable distances apart will be successfully pollinated, but might also increase gene flow. It has been assumed that male and female flowers had to be in anthesis at the same time in the same vicinity for successful pollination (MEIJER 1958, BEAMAN et al. 1988, NAIS 2001). Long-term pollen longevity is unusual but not exceptional: in *Pinus strobus* pollen remains viable for more than 15 months (DUFFIELD & SNOW 1941).

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Details of the pollination of *S. ram* (by females of at least 10 species of copro- and necrobiodotic *Sarcophaga*) and *S. poilanei* GAGNEPAIN emend. BÄNZIGER & HANSEN (by male and female *Lucilia papuensis* MACQUART) are in preparation, whereas that of *Ra. pricei* MEIJER, *Ra. kerrii*, *Rh. infanticida* and *Rh. deceptor* BÄNZIGER & HANSEN have already been treated (BEAMAN et al. 1988; BÄNZIGER 1991, 1996, 2001).

The seeds of Rafflesiaceae were traditionally assumed to be dispersed exozoochorously on the snout, feet and claws of squirrels, treeshrews, pigs (e.g. MEIJER 1958) and various other animals (e.g. ERNST & SCHMID 1913). In a detailed discussion, BOUMAN & MEIJER (1994) indicated zoochorous seed dispersal in the family but did not specifically implicate endo- or exozoochorous dispersal. EMMONS et al. (1991), however, after observing the squirrel *Callosciurus notatus* (BODDAERT) and the (non-rodent) treeshrew *Tupaia tana* RAFFLES eating a fruit of *Ra. keithii* in a forest in Borneo, argued that endozoochorous dispersal appears more important.

This study of *S. himalayana* presents further evidence for the latter. Namely, (i) significant amounts (average over 300) of intact (exotegmen unbroken) seeds are expelled per excrement pellet by rats fed on ripe fruit. (ii) The outer periclinal walls are removed, at least in part, in seeds that have passed through the digestive system (Fig. 3f). This is interpreted as an indication that digestion is required for, or at least facilitates, germination, a well-known feature in many plants. (iii) The seeds are very small and (iv) very tough (i.e., cannot be broken if rubbed between fingers), hence suitable to survive mammalian frugivory. These findings, and the type of dentition marks left on the fruits – two parallel line incisions – are all indicative of endozoochorous dispersal by medium-sized rats or squirrels. The number of seeds stuck on snout and claws must be small and further reduced by the tendency of rodents to clean their faces and feet after feeding, as can be easily observed in pets. The seeds carried on claws would soon be lost on the first substrate encountered. Thus they would most likely re-infect the same individual of *Tetrastigma* host that the parent rafflesias are already parasitizing. In endozoochorous dispersal there is a time lag of many hours between eating and excreting, during which rodents may wander hundreds of meters. The consequent smaller likelihood of being released at a *Tetrastigma* host may be compensated by the much higher number of seeds dispersed, some 15,000 from a single *Sapria* fruit and several times as many from a *Rafflesia*, in dozens of pellets over several hours.

Rats have never been seen consuming such fruit in nature and – with one exception – have never been proposed as their possible seed dispersers. ELLIOTT (1990) noted rodent tooth marks on the remains of 2.5 months old female flowers of *S. himalayana* in N Thailand. He attributed the marks to rats and squirrels, some of which he had seen and trapped in the area, and thought they may act as seed dispersers but did not elaborate whether this was endo- or exozoochorously. Unlike squirrels (except flying squirrels) and treeshrews, rats are nocturnal, the probable reason why they have not yet been seen consuming fruits of Rafflesiaceae. Furthermore, rats (*Rattus* spp.) are very species-rich, with about 22 species in Thailand and W Malaysia (MEDWAY 1969, MARSHALL 1988). Most are sylvatic and only three are synanthropic. Ground squirrels in the same area number only four species. An additional three mainly arboreal species visit the ground at some time, whereas the remaining c. 12 or so never or only exceptionally visit the ground (MEDWAY 1969, ASKINS 1988). The forest floor is a habitat more typical of rats than squirrels. By passing most of their lives on and below the ground, rats are also nearer the most infection-susceptible part of

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Rafflesiaceae's hosts: their root system. Roots, especially the widely ramified fine rootlets, are not protected by the thick corky bark of the stems of the *Tetrastigma* liana. By their burrowing activity, rats may additionally cause damage to roots through which the young parasite may penetrate the host, if a wound is at all necessary for infection. The ubiquitous presence of gnawing soil arthropods and nematodes may be just as efficient (BÄNZIGER 1991).

With manual pollination we now have an effective tool for improving the conservation of endangered Rafflesiaceae in two ways. One is to increase fruit set by manually pollinating flowers in suitable populations and let nature take care of subsequent steps. The other is to devise techniques to enhance host infection. However, for this it will be necessary to crack the mystery of how Rafflesiaceae infect their hosts. Manual pollination will play a fundamental role in providing seeds required for experimentation. The incurred drain in seed from the habitat can be easily compensated, thanks to the 6-10 fold higher fruit set obtained by manual pollination compared to natural pollination.

Zusammenfassung

Die reifen Früchte und Samen von *Sapria himalayana* und *Rafflesia kerrii* (Rafflesiaceae) werden zum ersten Mal beschrieben. Die Früchte der beiden Gattungen unterscheiden sich deutlich in Ontogenie, Form, Größe und Farbe. Sie sind mit Rissen durchsetzte Beeren, bei *S. himalayana* abgeflacht-fassförmig, braun-schwarz, 250-375 g schwer, 21-32 cm im Umfang und 3.1-5 cm lang, bei *R. kerrii* dagegen abgestutzt-kegelförmig, rot-braun, 40-51 cm im Umfang und 7.5-11.5 cm lang. Die Samen beider Gattungen sind ähnlich, winzig, "J"-förmig, "pockennarbig", 0.6-0.65 mm lang und 0.29-0.33 mm breit bei *S. himalayana*, ein Drittel grösser bei *R. kerrii*. Eine Methode zur Handbestäubung von *Sapria*, *Rafflesia* und *Rhizanthes* (einer weiteren Gattung der Rafflesiaceae) wurde entwickelt: nach einer Operation, bei der in einigen der Arten Teile der Blüte entfernt werden müssen, wird der Pollen mittels Aluminiumstreifen, die zum Erreichen der verborgenen Bestäubungsorgane zuvor zweckmäßig gebogen worden sind, von den Antheren aufgenommen und an die Stigmen abgestrichen. Die Fruchtbildung von *S. himalayana* wurde damit gegenüber den in Natur vorgefundenen 8-12% der Blüten auf 78% erhöht. Die eingeschlechtlichen Blüten sind nicht apomiktisch, sondern müssen bestäubt werden, um zu fruchten. Erstaunlicherweise wachsen die Ovar und Ovuli in nicht bestäubten Blüten während 4-5 Wochen nach der Anthese weiter, sind aber nach 6-9 Wochen abgestorben. Der Pollen, der als eine dickflüssige Suspension an den Antheren entlassen wird, koaguliert und erhärtet bald nach Aufnahme am Thorax des Bestäubers, wird aber schnell wieder verflüssigt, sobald er in Kontakt mit der Narbenflüssigkeit kommt. Erstarrter Pollen behielt drei Wochen lang die Fähigkeit, zu reifen Früchten und Samen zu führen. Bestäuber von *S. himalayana* waren die Goldfliege *Lucilia porphyrina* (WALKER), jene von *R. kerrii* fünf Arten von Schmeissfliegen, vor allem *Chrysomya villeneuvi* PATTON und *C. defixa* (WALKER) (Diptera, Calliphoridae). Die Entwicklung der Frucht und der Samen von *S. himalayana* wird von der Bestäubung bis zum natürlichen Aufspalten der reifen Frucht 5-6.5 Monate später dokumentiert. Alle nicht geschützten Früchte wurden im Reifestadium oder kurz davor von Nagetieren gefressen (wahrscheinlich Waldratten eher als Eichhörnchen), den vermutlichen Verbreitern der Samen. Laborratten, die in Experimenten eine ganze Frucht fraßen, schieden um die 15,000 unbeschädigte Samen im Kot aus. Daraus und aus weiteren Überlegungen wird gefolgert, dass bei *Sapria*, *Rafflesia* und *Rhizanthes* eine endozoochore Verbreitung der Samen wahrscheinlicher ist als eine exozoochore. Die Handbestäubung kann dank des damit ermöglichten, 6-10fach erhöhten Fruchtens zur Erhaltung dieser gefährdeten Rafflesiaceen verwendet werden.

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