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Fig wasps: mechanisms of pollen transfer in *Malvanthera* and *Pharmacosycea* figs (Moraceae)

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Abstract: Pollination of the pistillate fig flowers (*Ficus* spp.) has crucial effects for the figs and the pollinating wasps (Agaonidae). It allows normal development, of seeds and wasps. Some agaonid groups have evolved pockets and corbiculae to carry pollen, while others carry it in the intersegmental membranes and other hidden areas of the body. Single female specimens of *Pleistodontes rieki* Wiebes, *Robertsia mandibularis* Boucek, *Tetrapus americanus* Mayr and *Tetrapus* sp., the first two from New Guinea and the *Tetrapus* spp. from Costa Rica were studied with scanning electron microscopy. Some *Pleistodontes* species (Old World) have pollen cavities in the expanded scapes; they also carry pollen in the ridges and laminae of the mandibles. *Tetrapus* species (New World) carry pollen in cavities located behind the mandibular appendages and in the lamellae, ridges and teeth of the mandibles. These ways of carrying pollen may be intermediate between pollen transport in the intersegmental membranes, and in pockets or corbiculae. The evolution of such mechanisms may preserve pollen from desiccation. These structures may have similar functions to the pollinium of some plants that are also pollinated by specific insects. It is suggested that some fig wasps that become dusted with pollen in the syconia where they develop, and remove most of it once outside, fill up the "spurious" pockets, and other elaborate pollen-carrying structures outside the syconia. Abundant pollen grains were also found among the mouth parts of *Robertsia mandibularis* (Sycoecinae) a non-pollinating fig wasp.

Key words: Pollination, *Ficus,* fig wasps, Agaonidae, ultrastructure.

The Urticales are wind pollinated and successful adaptation to insect pollination is only known in *Ficus* (Moraceae) which has a pseudocarpous inflorescence (Berg 1977). According to Wiebes (1986) pollen transfer has only been studied in a small number of agaonid species, a situation which has not changed to the present.

The genus *Ficus* is exclusively pollinated by chalcidoid wasps of the family Agaonidae. The manners in which the minute, smooth and almost hairless wasps carry enough pollen to accomplish pollination and fertilization of the hundreds, if not thousands, of female flowers of the receptive syconia had been in doubt for a long time, until pollen pockets and corbiculae to carry pollen were found in some agaonid groups (Ramírez 1969, Galil and Eisikowitch 1969, Chopra and Kaur 1969). Other questions about the Agaonidae-*Ficus* association are why and how fig wasps transport pollen (Verkerke 1987). It is known that unpollinated syconia do not develop normally and that in the absence of pollination and fertilization of the "gall flowers" the nucelar tissue and the parthenocarpic endosperm, if present, is a poor food source for the wasp larvae and insufficient for the development of most females (Galil and Eisikowitch 1971, Galil 1973a). Ramírez (1969) noted that New World *Tetrapus* and some other Old World agaonids (e.g., *Pleistodontes*) carry pollen dusted over the body or in the digestive tract (see also Ramírez 1974), while according to Wiebes (1977), "in many agaonid species, pollen can sometimes be found in small quantities in several places of the body *e.g.,* between the mouth parts, and under the hypogidium, and that, in the species of *Pleistodontes* the axial, disto-dorsal excavation of the antennal scape may be such a place".

Galil and Eisikowitch (1971) stated that "undoubtedly, the wide fluctuation in number of anthers in the syconia of different *Ficus* spp., reflects on the efficiency of pollination". Wiebes (1982a) stated that nothing about pollination is known for the *Pleistodontes* species. The purpose of this article is to explain how some *Pleistodontes* wasps (the pollinators of section *Malvanthera*) and *Tetrapus* (the pollinators of section *Pharmacosycea*) carry the pollen and effect pollination. The presence of abundant pollen grains among the mouth parts of *Robertsia mandibularis* (Sycoecinae), a non-pollinating wasp, is also reported.

MATERIALS AND METHODS

One female specimen of each species studied was dissected, treated and mounted (Table 1).

The *Tetrapus* and *P. plebejus* wasps were originally preserved in 70% ethanol, while the *R. mandibularis* were pinned specimens. They were dissected under a stereoscope, cleaned, hydrated and inmersed in a fixative containing 2% glutaraldehyde and 1% formaldehyde in a 0.1 M Sorensen phosphate buffer solution for 1 hr. The wasps were post-fixed with buffered 1% osmium tetroxide for 2 hr and, after a brief rinse in distilled water and dehydration through a graded ethanol series, they were placed in isoamyl acetate, critical point-dried using CO_2, splutter-coated with platinum and observed in a scanning electron microscope at 15 kV.

TABLE 1

Wasps used for the SEM study

Wasp species	Geographic origin	Fig hosts
Pleistodontes plebejus Wiebes (1977)	New Guinea, West, River Tor	*Ficus xylosycea* Diels (1935)
Robertsia mandibularis Boucek (1988)	New Guinea, Manki area	*F. xylosycea*
Tetrapus americanus Mayr (1885)	Turrialba, Costa Rica	*F. maxima* Miller (1768)
Tetrapus sp.	San Isidro General, Costa Rica	*F. macbridei* Standley (1937)

RESULTS

In *P. rieki* females there is a large cavity with many pollen grains in the axial distodorsal areas of the antennal scapes (Fig. 1). Pollen grains were also found among the mandibular ridges and lamellae (Fig.2). *P. rieki* females do not have pollen pockets or corbiculae and the axial surface of the coxa is rather smooth (Wiebes 1977). It belongs to the *P. rieki* group (Wiebes 1991) characterized by the absence of pollen pockets and corbiculae. *P. rieki* is the pollinator of *F. xylosycia* of subseries *Hesperidiiformes*, a fig found in eastern New Guinea (Corner 1965), Bouganville Isl. and Papua (Wiebes 1991). *Robertsia mandibularis* (Sycoecinae), a non-pollinating sycophilous wasp (van Noort 1994), that also develops in the syconia of *F. xylosycia*, was found to have numerous pollen grains among the mouth appendages (Fig. 3).

Tetrapus females, the pollinators of *F. macbridei*, had pollen grains lodged in the mandibular lamellae (Fig. 4) and in a cavity (Fig. 5) on the rear part of the mandibular appendages. *Tetrapus americanus* females had pollen among the teeth of the mandibular appendage (Fig. 6). *Tetrapus* females of both species were also observed to eclose from the syconia completely covered with pollen, which they scraped from their bodies with the legs while standing on the syconia before flying away.

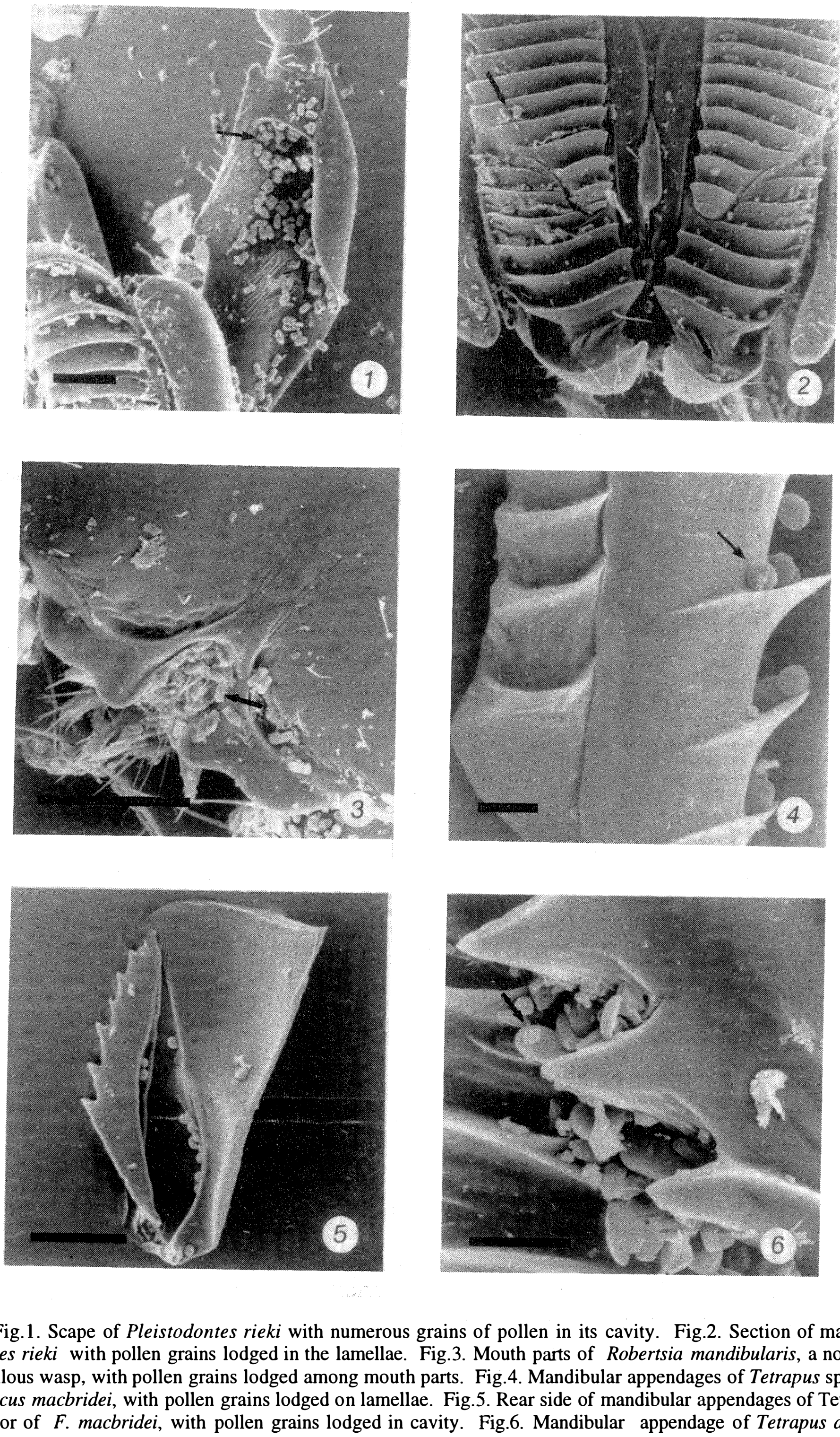

Fig. 1-6. Fig.1. Scape of *Pleistodontes rieki* with numerous grains of pollen in its cavity. Fig.2. Section of mandibles of *Pleistodontes rieki* with pollen grains lodged in the lamellae. Fig.3. Mouth parts of *Robertsia mandibularis*, a non-pollinating sycophilous wasp, with pollen grains lodged among mouth parts. Fig.4. Mandibular appendages of *Tetrapus* sp. the pollinator of *Ficus macbridei*, with pollen grains lodged on lamellae. Fig.5. Rear side of mandibular appendages of Tetrapus sp., the pollinator of *F. macbridei*, with pollen grains lodged in cavity. Fig.6. Mandibular appendage of *Tetrapus americanus* with pollen grains lodged among the serrations of the mandibular appendage.

Pollen in the scape of *Pleistodontes*: Most, if not all the *Pleistodontes* females, have the scape several times longer than wide, dorsoventrally depressed, with lateral and sometimes apical extensions (*e.g., P. rigisamos* Wiebes as illustrated by Wiebes (1991, Fig. 3). Grandi (1952) called the extension of the scape "lobo laminare de suo margine anteriore". The extension of the scape produces a flap over a large cavity in some of *Pleistodontes* females as illustrated for *P. rieki* (Fig. 1). Expanded scapes were also observed by Ramírez in *P. froggatti* Mayr, *P. blandus* Wiebes, *P. longicaudus* Wiebes, *P. mandibularis* Wiebes, *P. nigriventris* (Girault), and *P. rieki* Wiebes, at the Natural History Museum of London. The expanded scapes in other species of *Pleistodontes* may be used to carry pollen as in *P. rieki* and other wasps of the *P. rieki* and *rennellensis* groups (*sensu* Wiebes 1977). Since the agaonid pollinating females are short lived (Ramírez 1970) and do not feed as adults, the pollen grains observed on the "spurious pockets" of the wasps studied should be *Ficus* pollen.

Variety of locii in *Pleistodontes*: *Pleistodontes* wasps inhabit figs of the section *Malvanthera* (subg. **Urostigma**). *Malvanthera* figs are found in Australasia (Corner 1965, Berg 1989). The syconia of this group have numerous large ("explosive") anthers from which the pollen usually comes out naturally after the deshiscence of the anthers (Ramírez 1974), and as observed by Pemberton (1921) for *Ficus macrophylla* Desf., and the wasps emerge literally white with it; however, Pemberton (1921) did not mention wasp cleansing; they have filiform (bifid or simple) stigmata forming a synstigma. Pemberton (1921) observed that the eclosing *P. froggatti* Mayr females became dusted with pollen. Wiebes (1991) divided the genus *Pleistodontes* into several groups: the *P. rieki* and *P. rennellensis* groups, that do not posses pollen pockets and the *P. froggatti, P. nigriventris* and *P. imperialis* groups, which have them. In *Pleistodontes* we find a trend of pollen carrying mechanisms as follows: 1. Wasps with rather smooth mesosternum and axial surface of the coxa (e.g., *P. longicaudus, P. plebejus, P. inmmaturus* Wiebes, and probably *P. galvinus* Wiebes; 2. Wasps with an extensive field of setae on the axial edge of the fore coxa (*e.g., P. galvinus* and *P. regalis* Grandi); 3. *P. rennellensis* Wiebes, with a very vague trace of sternal pockets. 4. Wiebes (1977) illustrated *P. blandus* and described *P. mandibularis* with coxal corbiculae only. 5. Wasps with well developed mesosternal pockets and smooth mesosterna (e.g., *P. froggatti, nigriventris* and *imperialis* groups, (*sensu* Wiebes 1991). *P. imperialis* Saunders also have a coxal corbiculae (Ramírez 1974, 1978, Wiebes 1977). None of the wasps in categories 1-4 seem to have evolved conspicuous pockets or corbiculae to carry pollen, and they probably rely largely for pollination on the pollen carried in the cavities of the scapes as in *P. rieki* and other hidden areas. Thus, in *Pleistodontes* there are wasps that probably accomplish topocentric pollination (those without pockets or corbiculae) and those which accomplish ethodynamic pollination those with pockets and or corbiculae (for pollination syndromes, see Galil 1973 b). A similar trend is found in the pollinators of section *Conosycea* (subgenus *Urostigma*) although they do not carry pollen in the scapes (Ramírez 1974, 1978). The expanded scapes in other *Pleistodontes* species may be used to carry pollen as in *P. rieki* and *rennellensis* groups (*sensu* Wiebes 1977). *Pleistodontes* males do not possess expanded scapes.

Pollen transport in *Tetrapus*: *Tetrapus* wasps are the exclusive pollinators of the endemic New World *Pharmacosycea* figs (section *Pharmacosycea*). The syconia of this section have abundant long pedicellate male flowers, with two or three stamens with large "explosive" anthers with free long filaments. The anthers dehisce and shed the pollen without the help of the wasps (Ramírez 1970). *Tetrapus* females do not possess pockets, corbiculae or expanded hollow scapes to carry pollen, and become completely dusted with it when they eclose from the gall flowers. Once outside the syconium they remove most of the pollen with the frontal and hind legs. However, part of the pollen probably remains in some hidden body areas or spurious pockets (e.g., in a cavity between the mandibular appendages (Fig. 4) on the lamellae (Fig. 5) and the teeth (Fig. 6) of the mandibular appendages. *Tetrapus* wasps effect topocentric pollination as noted by Galil and Meiri (1981) for the pollination of the edible fig (*F. carica* L.) and by Berg

(1989, 1990) for section *Pharmacosycea.*

The eclosing *Pleistodontes* and *Tetrapus* females bocome completely dusted with pollen inside the syconia where they develop, and remove most of it with their legs from the body once outside the syconia; thus, the probability exists that they fill up with pollen the body depressions (spurious pockets *sensu* Galil and Neeman 1977) and pollen pockets while cleansing the body. Consequently "loading" of pollen may be not "passive". Galil and Neeman (1977) also observed that the emerging *Blastophaga psenes* L. (the pollinator of the edible fig *F. carica* L), a wasp without special structures to carry pollen, became abundantly covered with pollen and then commenced the prolonged task of cleansing their bodies from the surplus pollen once the wasps were outside the syconia (see also Galil and Meiri 1981).

Evolution of pollen transport: It is assumed that *Ficus* species with numerous male flowers and large anthers that open readily and widely, from which pollen comes out naturally, and is "passively" carried on the wasps' bodies, are more primitive than those with few male flowers from which the pollen is extracted from the anthers by the pollinating wasps (Ramírez 1974). The fig species with topocentric pollination usually have long branched bifid stigmata at least in the female flowers, usually forming a synstigmatic layer. The production of abundant pollen by species of figs which have pollinators without pollen pockets or corbiculae is extremely wasteful since most of the pollen is removed from the wasps' body by their cleansing movements after leaving the syconium (Galil and Meiri 1981). Filling of "spurious" pockets or other more elaborated structures outside the syconia may constitute another pollinating syndrome in *Ficus.*

Implications for pollinator systematics: *Pleistododes* and *Tetrapus*, as well as the pollinators of the African fig section *Galoglychia*, are considered to be related by Wiebes (1982b) and included in subfamily Agaoninae (see also Wiebes 1973, 1974, 1982 a, b and 1986). However, because of their pollination syndromes, their geographic distribution and the morphology of the head, these three agaonid groups do not seem to form a holophyletic group, as noted by Ramírez (1991). All known pollinators of *Galoglychia* figs have pockets and corbiculae to carry pollen and effect ethodynamic pollination. Because of the mode of pollination and some morphological characters (*e. g.*, presence of sensilla linearia in the fourth segment, non-breakage of antennae and wings upon entrance of the syconium and the absence of pockets and corbiculae to carry pollen), *Tetrapus* wasps seem to be the most primitive group of symbiotic agaonids and may form a subfamily apart. Section *Pharmacosycea* (the host of *Tetrapus*) also is considered a primitive fig group (Ramírez 1974, Berg 1990). That the pre-agaonids and the primitive agaonids probably carried the pollen in the mouth, and other natural cavities, as suggested by Ramírez (1974), is revealed by the presence of numerous pollen grains among the mouth parts of *Robertsia mandibularis* (Sycoecinae) a non-pollinating wasp. The way the agaonid symbiotic wasps carry pollen and the mode of pollination they effect may help to separate and establish the fig and agaonid taxa in more meaningful biological and ethological ways. Since the eclosing females of *Pleistodontes* and *Tetrapus* become completely dusted with pollen inside the syconia where they develop, and remove most of it with their legs from the body once outside the syconia, the probability exists that they fill up with pollen the body depressions (spurious pockets *sensu* Galil and Neeman 1977) and pollen pockets while cleansing the body. Consequently "loading" of pollen may be not "passive".

RESUMEN

La polinización de las flores pistiladas de los higos (*Ficus* spp.) tiene efectos positivos para los siconos y sus polinizadores agaónidos: permite el desarrollo normal de semillas y de las avispas; especialmente, el de las hembras. Algunas avispas polinizadoras de los higos evolucionaron sacos y corbículas poleníferos, mientras que otras llevan el polen en las membranas intersegmentales y otras áreas escondidas del cuerpo. Se informa que algunas especies de avispas *Pleistodontes* (Viejo Mundo) poseen cavidades para acarrear polen en los escapos y en los bordes y láminas mandibulares. Las avispas *Tetrapus* acarrean polen en cavidades localizadas detrás de los ápendices mandibulares y en los dientes y láminas de esos ápendices. Las formas de llevar pollen descritos parecen ser intermedios entre el transporte de polen en las membranas intersegmentales y aquella en sacos y corbículas. La evolución de cavidades, sacos y corbículas para llevar polen en los polinizadores de los higos parece ser mecanismos que evitan que los granos de polen se sequen cuando las avispas están buscando siconos receptivos para ovipositar, estas estructuras pueden tener función similares a aquellas de las políneas de algunos otros grupos de plantas. Abundantes granos de polen se encontraron también entre las piezas bucales de *Robertsia mandibularis* (Sycoecinae), una avispa que no es polinizadora.

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