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SHORT COMMUNICATION

# Evidence of orchid visitation by *Bactrocera* species (Diptera: Tephritidae) in Papua New Guinea

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*Bactrocera* Macquart (Diptera: Tephritidae) is a genus of nearly 500 species, the majority of which are endemic to tropical South-East Asia and Australasia (Drew 1989). Nearly all *Bactrocera* have frugivorous larvae that feed on the fleshy fruit of tropical trees, shrubs, and vines, and some species are major horticultural pests (White & Elson-Harris 1992). In this paper, where the term 'fruit fly' is used, we are referring explicitly to species of *Bactrocera*.

Males of many *Bactrocera* species respond positively to either one of two chemicals, 4-(4-acetoxyphenyl)-2-butanone (cue-lure) and 4-allyl-1,2-dimethoxybenzene (methyl eugenol or ME). Methyl eugenol is a naturally occurring chemical and is found in plants of up to ten different families (Metcalf *et al.* 1979). Cue-lure does not occur in nature, although a close chemical analogue,

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4-(4-hydroxyphenyl)-2-butanone (raspberry ketone), is found in three plant species (White 2000). Although known to fruit-fly workers since the 1910s (ME) and 1960s (cue-lure), the biological significance of these chemicals to fruit flies was not known until very recently (see for example Cunningham 1989).

In the last few years, evidence has come to light suggesting a biological use of these chemicals by fruit flies. As reviewed most recently by Shelly (2000), Tan & Nishida (2000) and White (2000), it is now regarded as probable that both ME and cue-lure are involved in the pheromone systems of dacine fruit flies; male flies fed on ME attract more females and have greater mating success than non-exposed flies. It is becoming more obvious, therefore, that natural sources of these chemicals are important, but to date largely neglected, components of the fruit fly's habitat.

While an association between mating behaviour and male lures seems well supported by existing experimental data, there is still little field evidence of how commonly wild flies search for natural sources of these chemicals, or how many *Bactrocera* species exhibit this behaviour. One likely natural source of both ME- and cue-lure-related chemicals, occurring in the rain-forest habitat of fruit flies, is orchids. Orchids of at least three genera are attractive to both ME- and cue-lure-responsive flies and, more importantly, flies may ingest orchid-derived chemicals (Nishida *et al.* 1993) and from this gain reproductive benefits (Tan & Nishida 2000). Tan & Nishida (2000) further argue that such visitations are of mutual benefit to the orchids as the flies may act as pollinators, although of this they do not present direct evidence. However, the number of *Bactrocera* species known to associate with orchids is still quite small (11, or ∼ 2% of described species) and is a total derived from across the entire geographic range of the genus. For no individual region or country do we have a comprehensive assessment of how many of the local fruit fly species may visit orchids.

White (2000) reported two cases of dead fruit fly specimens bearing orchid pollinaria, the massed pollen 'bundles' and their sticky basal attachment, that are carried by orchid pollinators (Dressler 1990, Nilsson 1992). Pollinaria are natural markers of insect visits to orchids, as there are no other obvious means by which they would be acquired by insects. Pollinaria have been used extensively in the euglossine bee/orchid work of South America (Dressler 1982, Roubik & Ackerman 1987) as evidence of bee visitation to orchids. Analysis of flies bearing pollinaria, therefore, offers an indirect means of assessing the occurrence of fruit fly visitation to orchids. Unfortunately though, in the absence of specialist local knowledge, species-level identification of the orchid pollinaria is difficult.

This paper reports on *Bactrocera* species bearing pollinaria that have been collected as part of a research programme currently underway in Papua New Guinea (PNG). PNG has the world's richest dacine fruit fly fauna, with 190 described species (Drew 1989); it also has an exceptionally diverse orchid flora

(Millar 1978). Given this diversity in both the fly and orchid fauna, PNG offers an opportunity to assess how common are orchid/fruit fly interactions.

Fruit flies were collected from June 1998 to September 1999. All provinces of PNG were sampled with the exception of Western, Gulf and Southern Highlands. Traps were hung, wherever possible, in fruit fly host trees at approximately 1.8 m above the ground. The survey covered four of the five broad agro-ecological zones recognized by PNG National Agricultural Research Institute, viz. Dry-lowlands, Wet-lowlands [mainland], Wet-lowlands [islands] and Highlands (1200–2000 m asl). The Dry-lowlands are typified by savanna and open-woodland; the Wet-lowlands (both mainland and islands) are typified by closed tropical rain forests; while the Highlands consist of sub-tropical/temperate grasslands in valleys surrounded by high-altitude rain forests. The fifth region, High-altitude highlands, was not sampled as fruit flies are rare at altitudes above 1800 m. In total, 124 pairs of cue-lure- and ME-baited modified Steiner traps (White & Elson-Harris, 1992: their Figure 1) were established across the four regions, primarily in agricultural areas or native forests. Traps were cleared of flies every 2–3 wk and these were subsequently forwarded to Griffith University, Brisbane, where they were identified to species using the keys in Drew (1989). Prof. R. A. I. Drew carried out confirmation of species.

At the time of identification, flies bearing orchid pollinaria were noted and for most specimens the locality of the pollinaria on the body was recorded.

Three hundred and thirty-eight individuals of 24 *Bactrocera* species were recovered bearing pollinaria (Table 1) from a total sample of 1 084 077 flies from 119 species. The species with individuals bearing pollinaria were amongst those most commonly trapped in the survey. Sixteen out of the 20 most frequently trapped species had at least one individual recorded as bearing pollinaria. All species with pollinaria-bearing individuals were members of the subgenus *Bactrocera* (*Bactrocera*), despite species from other dacine genera (i.e. *Dacus*) or other *Bactrocera* subgenera being trapped, often in large numbers. For example, *B.* (*Sinodacus*) *triangularis* (Drew) and *B.* (*Zeugodacus*) *cucurbitae* (Coquillett) were ranked five and six respectively in the overall number of flies caught, but we recovered no individuals bearing pollinaria.

While the most abundant *Bactrocera* (*Bactrocera*) species were all represented by pollinaria-bearing individuals, the number of flies with pollinaria was not always proportional to the total number of individuals of a species in traps (Table 1). For example, *B. (B.) umbrosa* (F.) individuals represented only 13% of all flies caught, but represented 39% of the individuals with pollinaria. In contrast, *B. (B.) frauenfeldi* (Schiner) was the most abundant fly species, representing 28% of total collections, but only 4% of pollinaria-carrying flies were of this species. Equal numbers of ME and cue-lure species were recorded with pollinaria, but ME-responding flies (n = 282 individuals) were collected far more frequently than cue-lure flies (n = 56 individuals) (Table 1).

While individuals with pollinaria were trapped in most areas of PNG where

Table 1. Records of *Bactrocera* (*Bactrocera*) species bearing orchid pollinaria, collected from male lure traps in Papua New Guinea. 'Total flies collected' is the total number of flies of that species collected during the sampling period (June 1998–Sep 1999). The 'Rank order of fly species' is their ranked position on a list of 119 species based on their abundance in traps, with '1' being the most abundant.

| Species | Lure-type | Total flies collected | Rank order of fly species | No. with pollinaria | Location of pollinaria: thorax/abdomen | Province(s) where flies bearing pollinaria were collected |
|---|---|---|---|---|---|---|
| *B. (B.) umbrosa* | ME | 145 871 | 3 | 131 | 112/1 | East Sepik, Morobe, West New Britain |
| *B. (B.) curvifera* | ME | 25 217 | 8 | 54 | 46/0 | East Sepik, Morobe |
| *B. (B.) musae* | ME | 228 931 | 2 | 43 | 34/0 | Morobe |
| *B. (B.) papayae* | ME | 17 368 | 10 | 17 | 10/0 | East Sepik, Morobe |
| *B. (B.) atramentata* | Cue | 6 075 | 14 | 13 | 0/11 | East New Britain (ENB), New Ireland (NI) |
| *B. (B.) frauenfeldi* | Cue | 308 487 | 1 | 13 | 3/7 | Central, Madang, Morobe, ENB, NI |
| *B. (B.) fulvicauda* | ME | 8 943 | 12 | 12 | 11/0 | Morobe |
| *B. (B.) seguyi* | ME | 2 793 | 20 | 9 | 8/0 | Morobe |
| *B. (B.) bryoniae* | Cue | 86 519 | 4 | 8 | 2/5 | Central, Morobe |
| *B. (B.) moluccensis* | Cue | 21 648 | 9 | 6 | 0/4 | Madang, Morobe, ENB, NI |
| *B. (B.) trivialis* | Cue | 11 409 | 11 | 6 | 5/0 | Central |
| *B. (B.) dapsiles* | ME | 46 209 | 7 | 4 | 3/0 | Morobe |
| *B. (B.) bancroftii* | ME | 1 086 | 26 | 3 | 3/0 | Morobe |
| *B. (B.) lineata* | Cue | 4 822 | 15 | 3 | 0/2 | Morobe |
| *B. (B.) morula* | Cue | 741 | 31 | 2 | 0/2 | ENB |
| *B. (B.) aterrima* | Cue | 192 | 50 | 1 | – | ENB |
| *B. (B.) endiandrae* | ME | 4 384 | 18 | 1 | 1/0 | Morobe |
| *B. (B.) enochra* | Cue | 860 | 29 | 1 | – | Morobe |
| *B. (B.) nigrescens* | ME | 4 764 | 16 | 1 | 1/0 | West New Britain |
| *B. (B.) paramusae* | Cue | 2 513 | 22 | 1 | 0/1 | Morobe |
| *B. (B.) tinomiscii* | Cue | 910 | 27 | 1 | 0/1 | Morobe |
| *B. (B.) unistriata* | ME | 8 709 | 13 | 1 | – | Morobe |
| *B. (B.)* sp. nr *musae* | ME | – | – | 6 | 6/0 | East Sepik, Morobe |
| *B. (B.)* sp. nr *breviaculeus* | Cue | – | – | 1 | 1/0 | Central |

sampling occurred (Table 1), catches were not random across the trapping array. No flies with pollinaria were recorded from the PNG Highlands (altitude 1200–2000 m asl; region covered by Western Highlands, Chimbu and Eastern Highlands Provinces), while a disproportionately large number of flies with pollinaria were collected from Morobe Province (part of the Wet-lowlands mainland region) (83% of flies with pollinaria, 40% of total flies sampled from pollinaria-bearing species).

Location of pollinaria on a fly's body was clearly differentiated depending on whether the fly was a ME- or a cue-lure-responding species (Table 1). For the ME species, with only one exception, pollinaria were always located on the mesothorax of the fly. For most (77%) cue-lure flies pollinaria were placed on terga II-IV of the abdomen. *Bactrocera (B.) trivialis* (Drew) was unusual amongst the cue-lure flies in that all individuals recorded were carrying pollinaria on the thorax (Table 1). If this species is removed from the calculation, then 85% of cue-lure-responding flies carried the pollinaria on their abdomen.

For three fly species (*B.* (*B.*) *curvifera* [Walker], *B.* (*B.*) *musae* [Tryon], *B.* (*B.*) *umbrosa*) from Morobe Province, correlations were made of the monthly total of pollinaria-bearing flies against the total monthly catches of those species in the province. For all three species, there were significant correlations (df = 11; *B. curvifera* r = 0.86, $P < 0.01$; *B. musae* r = 0.83, $P < 0.01$; *B. umbrosa* r = 0.62, $P < 0.05$) (Fig. 1). This suggests that the catch of flies with pollinaria was related to total population size rather than being influenced by factors specific to the fly/orchid interaction (e.g. orchid flowering times). However, what is reinforced by these data, is the very low percentage of flies in the total trapped population that bore pollinaria.

We were unable to get the pollinaria on most flies identified. Under light microscopy all pollinaria were morphologically similar whether on the thorax or abdomen and could not be separated into different groups (attempted independently by the senior author and H. Proctor, Griffith University). Two pollinaria on the thoraces of *B. umbrosa* specimens were identified as *Bulbophyllum tollenoniferum* J. J. Sm. by Mr P. Spence (formerly Orchid Research Centre, Port Moresby) and three of the authors (A. R. C., D. P. & S. S.) personally observed four male *B. frauenfeldi* come to cut *Bulb. tollenoniferum* flowers. According to Spence (pers. comm.), flowering individuals of this orchid routinely attract hundreds of flies that crowd around the petals but rarely enter the flower. Millar (1978) presents illustrations of dacine fruit flies on the flowers of this species and another PNG species, *Bulbophyllum macranthum* Lindl. N. H. S. Howcroft (*pers. comm.* and Howcroft 1983, 1987) considers that all PNG species of the *Bulbophyllum* section Sestochilus (Brada) Benth. and Hooker f. (= sect Stenochilus) are attractive to, and pollinated by, *Bactrocera* species. The PNG species in this section are *B. baileyi* F. Muell., *B. caryophyllum* J. J. Sm., *B. gerlandianum* Kranzl., *B. gjellerupli* J. J. Sm., *B. grandifolium* Schltr., *B. guttatum* Schltr., *B. hahlianum* Schltr., *B. macranthoides* Schltr., *B. macranthum*, *B. tollenoniferum*,

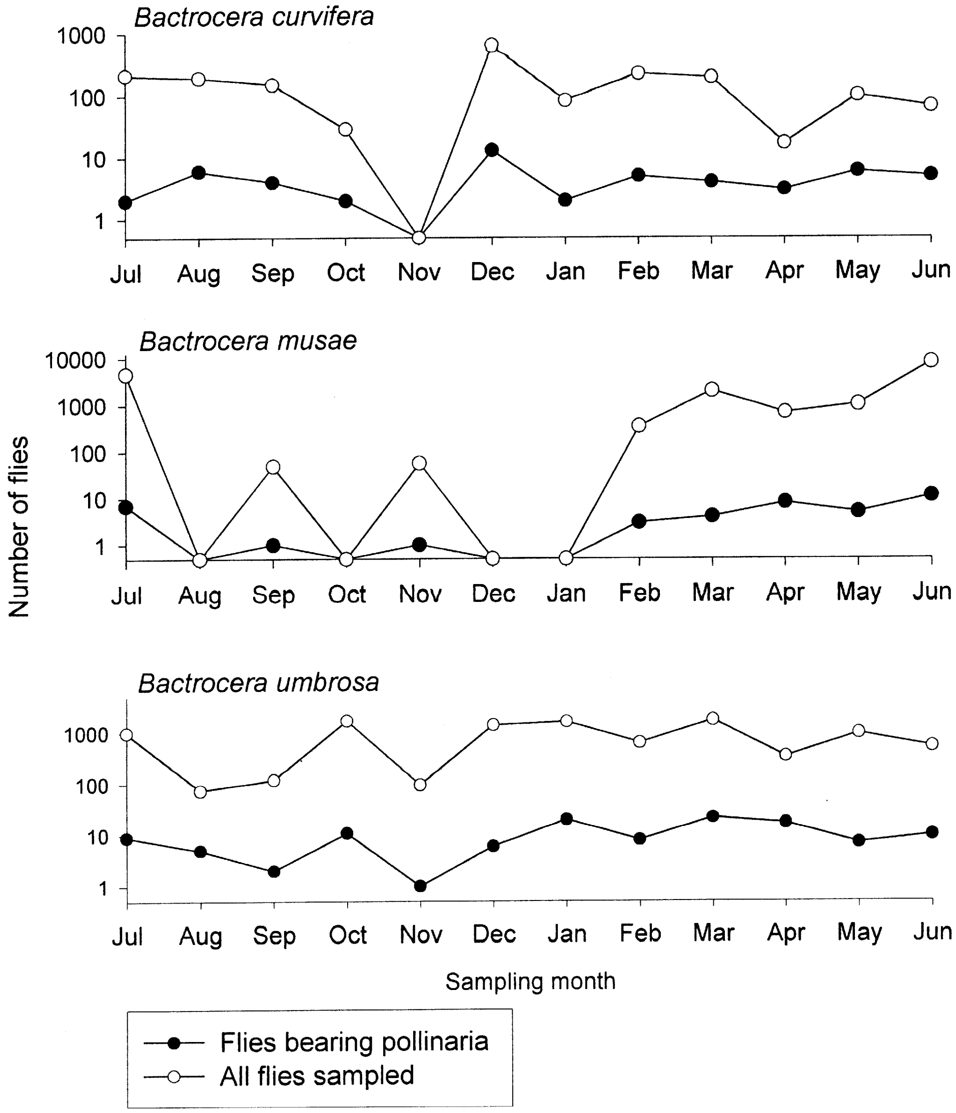


Figure 1. Total monthly catches of three *Bactrocera* species in male lure traps in Morobe Province, Papua New Guinea, 1998-99. 'All flies sampled' includes both flies with and without orchid pollinaria on their bodies.

*B. tortum* Schltr., *B. truncicola* Schltr. and *B. werneri* Schltr. (Howcroft 1983, 1987). Flowers of *B. baileyi* have been reported as attracting male *Bactrocera* in north Queensland (May 1953, Smythe 1969).

Using pollinaria as a marker of orchid visitation, this paper identifies a further 21 species of dacine fruit fly known to respond to orchids, nearly double the number previously known. More importantly, of the 20 most abundant species caught in our trapping system, all were represented by pollinaria-bearing individuals with the exception of three non-*Bactrocera* (*Bactrocera*) species and *B.* (*B.*) *neohumeralis* (Hardy) (ranked 17). Below this top 20, it is probable that the relatively small samples sizes (less than 1000 individuals) meant that by chance we did not collect pollinaria-bearing flies, as catching such flies was a rare event. For three species where we had sufficient numbers to check, those individuals bearing pollinaria were a constant, albeit extremely low, proportion of the total population. These two lines of evidence suggest that visitation to orchids is a consistent, but possibly uncommon behaviour across and within *Bactrocera* (*Bactrocera*) species.

Representing less than 0.05% of the total catch, flies with pollinaria were rare in collections. The issue of concern is how accurately this reflects fruit fly visitation to orchids? That is, is fly visitation truly a rare event, or is relying on catching pollinaria-bearing individuals a poor measure of a more common event? Although only direct observation will provide a conclusive answer, we suspect that many more flies visit orchids than are subsequently caught bearing pollinaria. While a single blossom may attract many flies over several days (Tan & Nishida 2000), each blossom will produce only two or four pollinaria, depending on the species (Dressler 1990) (assuming that the orchids involved are species of *Bulbophyllum* and/or *Dendrobium*, as suggested by our observations and previous literature records). Thus, only a small percentage of the many visiting flies will have the potential to pick up the marker.

In suggesting this view, we do not wish to imply that we consider every individual fruit fly visits orchids. Other sources of these chemicals occur in nature and foraging flies may feed on these (Nishida *et al.* 1997, Shelly 2000, Yong 1992). Thus, some proportion of a fruit fly population may not visit orchids if other plant species are available.

While this paper has focused on methyl eugenol and cue-lure/raspberry ketone as the chemicals which flies may be utilizing in orchids, it is possible that alternative chemicals may be driving the fruit fly–orchid interaction. Tan & Nishida (2000) reported that zingerone, the pungent essence of ginger, was the chemical responsible for attracting both ME- and cue-lure-responding dacine fruit flies to the orchid *Bulb. patens* King. In PNG, *Bulb. tollenoniferum* similarly attracts flies of both lure types as pollinaria on *B. umbrosa* (an ME fly) was identified as belonging to this species, while we directly observed *B. frauenfeldi* (a cue-lure fly) coming to flowers of this species.

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