

Table 2. Antiviral activity of MTA, xylo-MTA and xylo-A

Compound	aED <sub>90</sub>		bED <sub>50</sub>	
	HIV-1	HSV-1 μM	VV	ASFV
MTA	>20	>16	>16	>16
xylo-MTA	>190	>100	>100	>100
xylo-A	>5	0.7	0.4	0.1

aED<sub>90</sub> (Effective Dose 90): concentration of a compound required to reduce by 90% the HIV-1 yield. Virus yield in untreated controls was  $2.7 \times 10^5$  CCID<sub>50</sub> per mL. bED<sub>50</sub> (Effective Dose 50): concentration of a compound required to reduce the number of plaques by 50%. Number of plaques in untreated cultures was: 130 (HSV-1), 140 (VV), 145 (ASFV).

than MTA and xylo-A. None of the compounds showed a selective activity against fungi and bacteria, their MICs being always greater than the respective MNTDs for Vero and C8166 cells. When tested for antiviral activity, neither xylo-MTA nor MTA inhibited the multiplication of human immunodeficiency virus (HIV-1) or that of the other viruses at concentrations non-toxic for uninfected cells. By contrast, xylo-A was totally inactive against HIV-1 but showed a potent and selective activity against herpes simplex type 1 (HSV-1), vaccinia (VV) and African swine fever virus (ASFV). Selectivity indices (ratio MNTD Vero/ED<sub>50</sub>) were 10, 18 and 70, respectively.

The lower cytotoxicity of xylo-MTA with respect to MTA could explain the conversion of MTA into a less toxic metabolite which can be safely accumulated in the egg mass of the mollusc.

However, the evidence presented here does not support the idea of a protective role of xylo-MTA as such.

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## Oriental orchid (*Cymbidium pumilum*) attracts drones of the Japanese honeybee (*Apis cerana japonica*) as pollinators

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**Abstract.** The discovery that drones of the Japanese honeybee (*Apis cerana japonica*) pollinate the oriental orchid (*Cymbidium pumilum*) is reported. Drones are attracted to the orchid flower aroma mainly during their mating flights in April through May. Some drones cluster on the flower racemes and others insert their heads deep into the flowers. Drones with pollinia on their scutellum visit other orchids, which facilitates pollination. Individual workers and swarming colonies are also strongly attracted by the flower aroma, but the allopatric western honeybee (*Apis mellifera*) is not attracted.

**Key words.** Drone; male honeybee; pollination; *Cymbidium pumilum*; *Apis cerana japonica*.

The pollination strategy by which some groups of orchids attract particular bees or wasps by mimicking their sex attractants is well known as a fascinating example of insect-plant coevolution<sup>1</sup>. A similar relationship may have developed between the oriental orchid (*Cymbidium pumilum* Rolfe) and the oriental honeybee (*Apis cerana japonica*) as pollinators of this orchid before 1986, but in the spring of 1988 we also noticed that many drones were

attracted to the flowers of a potted orchid placed near an apiary of *A. cerana japonica* at Tamagawa University, Tokyo. Around noon, before the time of normal mating flights, some drones had already been attracted and were flying around or landing intermittently on the flowers or other parts of the plant. Some of them inserted their heads and thoraxes deep into the flowers between the column and lip (fig. 1). During the efforts of these drones to escape from being trapped, by using their middle legs,



Figure 1. Drone of *Apis cerana japonica* pollinating *Cymbidium pumilum*.

Figure 2. Pollinia of *Cymbidium pumilum* attached to scutellum of drone.



Figure 3. Drone aggregation on flowers of *Cymbidium pumilum*.

pollinia together with the pollinial cap stuck to their scutella just as they do with workers. Usually, the cap dropped away after a few seconds (fig. 2). The drones

then visited other orchid flowers and pollination took place although the frequency was lower than that of workers. Trophallaxis between drones, or drones and

workers, was also observed on the flowers. In contrast to drones, workers visit this orchid throughout the day and pollinate it.

During the first half of the mating-flight time, 13.00–15.00, many more drones visited the flowers and sometimes small clusters were formed (fig. 3). That the attraction is caused by a volatile substance(s) from the flowers is evidenced by the observations that: (1) plants without flowers have no attractivity; (2) flowers covered with black cloth retain their attractivity, and (3) an ether extract of an absorbent (Tenax TA) containing flower aroma (50 flowers/h equivalent) is highly attractive. When we carried the potted orchid away, a few drones followed for up to 100 m. Also, we found 9 dead drones beneath the orchid.

Subsequent seed production was confirmed and no other insect species visiting the flower was seen during three seasons of careful observation, which indicates that *A. cerana japonica* is the major pollinator of this orchid. Orchid flowers also attract swarming colonies<sup>2</sup>. We observed that swarming workers behaved differently from single visitors. It is emphasized that this orchid only attracts the oriental honeybee but not the western species. This indicates that the attractive principle(s) is not a known major constituent(s) of queen pheromones, such as 9-keto-2-decenoic acid or the Nasonov (aggregation) pheromone, or a mimic of such a substance, because the

major constituents of queen pheromones are thought to be common to both species.

This orchid has no intrafloral nectary; extrafloral nectaries do secrete nectar but *A. cerana japonica* rarely collects it, so there is no reward from the flowers. Thus, there is no benefit for the honeybees – in fact, the flower sometimes disturbs the normal reproductive swarming process by trapping the majority of the colony members. By contrast, for the orchid, repeated visiting by workers and drones results in crucially-important pollination.

The fascinating relationship between this orchid and *A. cerana japonica* is a new observation that might lead to the discovery of *A. cerana*-specific semiochemicals. The availability of such specific substances might assist in better management of the oriental honeybee, which shows strong absconding tendencies. Chemical analysis of the orchid aroma is now in progress.

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