Selbyana 5(3-4): 286-290, 1981

FLORAL FRAGRANCES IN *ANGRAECUM* (ORCHIDACEAE)

James T. Murrell,[1] *Norris H. Williams,*[2] *Alec M. Pridgeon,*[2]
and Calaway H. Dodson[3]

Extensive research on orchid fragrance components and their function both as pollinator attractants and as ethological isolating mechanisms has been carried out (Hills et al., 1968, 1972; Dodson, 1962; Dodson et al., 1969; Dressler, 1968; Williams and Dodson, 1972). Selective attraction, often species-specific, of male euglossine bees to various orchid flower fragrances has been confirmed by observing and collecting bees drawn to pads saturated with pure samples of floral fragrance components throughout Central America and northern South America (Adams, 1968; Dodson et al., 1969; Williams & Dodson, 1972). Gas chromatographic analyses of fragrances have shown that for every *Catasetum* species known to have a species-specific pollinator, the flowers of those species produce a species-specific fragrance, and that species of *Catasetum* which share identical floral fragrances attract the same pollinators (Hills et al., 1972). Such differential pollinator attraction by quantitative and/or qualitative variation in fragrance components can act as an isolating mechanism between sympatric species. Ethological isolation is often supplemented and reinforced by geographical, mechanical, temporal or seasonal, and/or ecological isolating mechanisms (Hills et al., 1972). Earlier investigations with gas chromatography have shown that species of the Old World tropical genus *Angraecum* Bory also produce species-specific fragrances, though the pollinators are not euglossine bees (which are confined to the neotropics) but night-flying moths (Dodson et al., 1969) This report will detail fragrance components in five *Angraecum* species and in two well-known intrageneric hybrids.

Angraecum consists of approximately 200 species distributed over tropical Africa and the surrounding islands (Piers, 1968). It is characterized by an epiphytic, monopodial habit; distichous, elliptic or strap-shaped leaves; lateral, racemose (rarely solitary-flowered) inflorescences; green to white, star-shaped flowers; a deeply concave lip which is attenuated apically and narrowed into a nectariferous spur at the base (Piers, 1968). The spur of *Angraecum sesquipedale* Thours is frequently as long as 35 cm, a phenomenon which prompted Darwin (1862) to investigate the pollination mechanism of the species and suggest that the agent was a night-flying moth with a proboscis long enough to reach the nectar at the tip of the spur. *Angraecum* species included in this gas chromatography study are: the three closely allied species *A. eburneum* Bory native to Madagascar and the Comoro Islands, *A. eichlerianum* Kranzl. ranging over tropical West Africa, *A. girymae* Rendle from Kenya, Tanganyika, and Zanzibar, and two distantly allied species *A. magdalenae* Schltr. and Perr. and *A. sesquipedale*, both known only from Madagascar. The two intrageneric hybrids examined are *A.* × Orchidglade (*A. girymae* × A. *sesquipedale*) and *A.* × Veitchii (*A. eburneum* × *A. sesquipedale*).

MATERIALS AND METHODS

Gas chromatography equipment and procedures followed those outlined in Hills et al. (1972) with few exceptions. Flowers of *Angraecum* taxa were sampled not in the morning as for most other genera but in the evening

[1]Department of Biological Sciences, Mississippi University for Women, Columbus, Mississippi 37901.
[2]Department of Biological Science, Florida State University, Tallahassee, Florida 32306.
[3]The Marie Selby Botanical Gardens, 800 S. Palm Avenue, Sarasota, Florida 33577.

between 6 p.m. and 6 a.m. which corresponded to the period of maximum fragrance production and to the time of presumed visitation by pollinators in their natural habitat. All *Angraecum* flowers were sampled with Carbowax 20M columns at 70, 130 and 160° C. As before, floral fragrance com-

Peak Number	Relative Retention Time	*A. eburneum*	*A. eichlerianum*	*A. girymae*	*A. magdalenae*	*A. sesquipedale*	*A.* × Orchidglade	*A.* × Veitchii
1	0.27	--	--	0.2	--	--	--	--
2	0.32	--	--	0.1	--	--	--	--
3	0.35	--	--	0.2	--	--	--	--
4	0.43	0.3	--	--	--	0.7	0.8	0.3
5	0.48	--	--	0.3	--	--	--	--
6	0.52	0.2	--	--	--	--	--	--
7 alpha-pinene	0.63	0.2	0.4	0.5	0.3	0.6	--	3.0
8	0.78	0.2	--	--	t	0.7	0.3	1.8
9	1.14	0.1	--	2.7	--	0.8	0.3	2.3
10	1.20	--	--	--	t	--	--	--
11 alpha phellandrene	1.41	0.2	--	--	0.1	--	--	--
12	1.63	--	--	--	--	--	0.2	--
13	1.70	0.1	--	--	--	1.3	--	1.8
14 1,8-cineole	1.79	--	--	0.3	--	--	--	--
15	2.23	1.6	--	--	--	--	--	0.7
16	2.38	--	0.8	0.2	--	--	--	--
17	2.81	--	--	0.3	--	--	--	--
18	0.26	--	--	1.4	--	10.6	--	--
19	0.33	--	--	1.8	--	--	--	--
20 citronellal	0.43	4.5	--	4.7	--	5.0	3.2	11.7
21 linalool	0.52	87.6	21.4	10.0	97.8	18.4	2.2	32.6
22	0.74	--	--	--	--	11.6	--	--
23 methyl benzoate	0.84	--	77.5	11.1	--	7.3	9.4	40.7
24	0.94	1.4	--	--	--	--	--	--
25 benzyl acetate	1.27	2.9	--	25.9	--	--	20.0	4.6
26 methyl salicylate	1.58	--	--	--	--	13.5	--	--
27 2-phenylethyl acetate	1.84	--	--	3.7	--	--	--	--
28	2.33	--	--	2.0	--	--	--	--
29	2.75	--	--	1.6	--	--	--	--
30	0.45	--	--	--	--	--	63.6	--
31	0.54	--	--	1.1	--	--	--	--
32	0.74	--	--	3.1	--	--	--	--
33	0.85	--	--	--	--	1.8	--	--
34	0.94	--	--	--	--	35.4	--	--
35	1.01	--	--	29.0	--	--	--	--
36	1.31	0.7	--	--	--	--	--	--
37	2.28	--	--	--	1.7	--	--	--

TABLE 1. Percentages of fragrance components in the injected samples.

ponents were identified by a comparison of relative retention times of known floral fragrance compounds used as standards to the relative retention times of the unknown compounds. Components of each fragrance were quantified by the integrator trace and calculated as a percentage of the injected sample. Compounds making up less than 0.1 percent of the fragrance were listed as trace (t) quantities.

Voucher specimens for all taxa have been deposited at the University of Miami herbarium (BUS).

RESULTS

Fragrance composition for each of the taxa sampled is listed in Table 1. All taxa but *A.* x Orchidglade have alpha-pinene as a very minor component of the fragrance and linalool as a significant but highly variable component. Linalool makes up as little as 2.2 percent of the total fragrance in *A.* × Orchidglade and as much as 97.8 percent in *A. magdalenae*. It is also the major peak in *A. eburneum. Angraecum eichlerianum* possesses a fragrance dominated by methyl benzoate, which with linalool accounts for approximately 98 percent of the fragrance. The fragrance of *A. girymae* is unique in several respects. First, it produced more compounds than any other species. Though most compounds represent a very small percentage of the total fragrance (such as peaks 1, 2, 3, 5, 14, 17), they are present only in *A. girymae*. It is the only species with 1,8-cineole as a fragrance component. Over half of its fragrance is composed of benzyl acetate and unnamed peak 35. *Angraecum sesquipedale* is the only species possessing methyl salicylate and compound 34, which together account for almost half of its fragrance.

Very interesting inheritance patterns appear in the fragrances of the two hybrids. Tables 2 and 3 compare selected peaks from Table 1 for each hybrid and its parentage. Table 2 shows that the fragrance of *A.* × Orchidglade derives compounds 4 and 8 only from the *A. sesquipedale* parent and benzyl acetate only from the *A. girymae* parent. The hybrid fragrance shares compounds 9, citronellal, linalool, and methyl benzoate with both parents. The relative percentages of compounds in the hybrid are generally lower than those in either parent, although the percentage of methyl benzoate in the hybrid is intermediate between those of the parents. Approximately 64

Peak Number	Relative Retention Time	*A. girymae*	*A. sesquipedale*	*A.* × Orchidglade (*A. girymae* × *A. sesquipedale*)
4	0.43	--	0.7	0.8
8	0.78	--	0.7	0.3
9	1.14	2.7	0.8	0.3
20 citronellal	0.43	4.7	5.0	3.2
21 linalool	0.52	10.0	18.4	2.2
23 methyl benzoate	0.84	11.1	7.3	9.4
25 benzyl acetate	1.27	25.9	--	20.0

TABLE 2. Floral fragrances of *A.* × Orchidglade and its parents.

percent of the fragrance of *A.* × Orchidglade is represented by compound 30, which is lacking in both parents and in other *Angraecum* species. Fragrance inheritance of *A.* × Veitchii is shown in Table 3. This hybrid possesses significantly larger percentages of alpha-pinene and citronellal than either parent. The same is true for peaks 8, 9 and 13. *Angraecum* × Veitchii derives peak 15 and benzyl acetate from *A. eburneum*, but methyl benzoate (40.7 percent) from *A. sesquipedale*.

DISCUSSION

Results from these preliminary studies stongly indicate that species of *Angraecum* produce species-specific fragrances which could serve as isolating mechanisms. In addition to the different proportions of linalool, methyl benzoate, and benzyl acetate which result in distinct fragrances, the presence of alpha-pinene in all species may further act as a modifier of the general fragrance (Adams, 1968). Little information on pollinator specificity in *Angraecum* is available, chiefly because of the problems associated with observing nocturnal pollination (Dodson et al., 1969). The genus is known to be sphingophilous, pollinated by hawk-moths which are strong fliers (Pijl & Dodson, 1966). The hawk-moths are then presumably able to travel long distances along fragrance concentration gradients and find the plants over fairly large distances. It is possible that the moths might bring about long distance gene flow in the same way as euglossine bees (Williams & Dodson, 1972).

A proportion of methyl benzoate in *A.* × Orchidglade intermediate between that in its parents suggests some degree of genetic complementation. However, this is not the case in other fragrance compounds of the hybrid. Also inexplicable (from such limited data) is the high percentage of methyl benzoate in *A.* x Veitchii.

ACKNOWLEDGEMENTS

Portions of this work were supported by a post-doctoral fellowship to

Peak Number	Relative Retention Time	*A. eburneum*	*A. sesquipedale*	*A.* × Veitchii (*A. eburneum* × *A. sesquipedale*)
4	0.43	0.3	0.7	0.3
7 alpha-pinene	0.63	0.2	0.6	3.0
8	0.78	0.2	0.7	1.8
9	1.14	0.1	0.8	2.3
13	1.70	0.1	1.3	1.8
15	2.23	1.6	--	0.7
20 citronellal	0.43	4.5	5.0	11.7
21 linalool	0.52	87.6	18.4	32.6
23 methyl benzoate	0.84	--	7.3	40.7
25 benzyl acetate	1.27	2.9	--	4.6

TABLE 3. Floral fragrances of *A.* × Veitchii and its parents.

JTM through NIH Training Grant NIH HD00187 to the Laboratory for Quantitative Biology, University of Miami, Coral Gables, Florida.

LITERATURE CITED

Adams, R. M. 1968, The attraction of Euglossini (Hymenoptera: Apidae) to fragrance compounds of orchid flowers. Ph.D. Dissertation. University of Miami. Coral Gables, Florida.

Darwin C. 1862. The fertilisation of orchids by insects. Murray, London.

Dodson, C. H. 1962. Pollination and variation in the subtribe Catasetinae (Orchidaceae). Ann. Missouri Bot. Gard. 49: 35-56.

Dodson, C. H., R. L. Dressler, H. G. Hills, R. M. Adams, & N. H. Williams. 1969. Biologically active compounds in orchid fragrances. Science 164: 1243-1249.

Dressler, R. L. 1968. Pollination by euglossine bees. Evolution 22: 202-210.

Hills, H. G., N. H. Williams, & C. H. Dodson. 1968. Identification of some orchid fragrance components. Amer. Orchid Soc. Bull. 37: 967-971.

———————————— 1972. Floral fragrances and isolating mechanisms in the genus *Catasetum* (Orchidaceae). Biotropica 4: 61-76.

Piers, F. 1968. Orchids of East Africa. Verlag von J. Cramer, Lehre, West Germany.

Pijl, L. van der & C. H. Dodson. 1966. Orchid flowers: their pollination and evolution. University of Miami Press, Coral Gables, Florida.

Williams, N. H. & C. H. Dodson. 1972. Selective attraction of male euglossine bees to orchid floral fragrances and its importance in long distance pollen flow. Evolution 26: 84-95.