

THE POTENTIAL SEED PRODUCTIVITY OF ORCHID FLOWERS AND PECULIARITIES OF THEIR POLLINATION SYSTEMS¹

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ABSTRACT: The relationships between potential seed productivity, pollinia structure, methods of pollen reception, the sizes of the stigma, and the visitation frequency of orchid flowers were studied. The method of pollen reception by the stigma has a more dominant influence on the ovule number than the structure of the pollinia. Although *Cephalanthera* species investigated have the most primitive pollinia structure among species studied, their number of ovules is higher than in *Orchis soriophora*, *Listera ovata*, *Dactylorhiza romana*, *Orchis tridentata*, *Orchis simia*, *Platanthera bifolia*, *P. chlorantha*, and *Steveniella satyrioides*. A larger part of soft pollinia of *Cephalanthera* is scraped by the rostellum of one flower from pollinators ("scraping" method). With the "contact" method of pollen reception (*Listera* and *Orchidinae* species) only the apical part of the pollinia comes in contact with stigmatic secretion, and a small amount of pollen of the pollinia is delivered onto the stigma. Therefore, the species with a scraping method have lower pollen/ovule (P/O) ratios than those with a contact method. In each method of pollen reception the ovule number and P/O ratio depend on the consistency of pollinia. The ovule number is lower in species with soft pollinia than in those with sectile or hard ones. The P/O ratio, on the contrary, is higher. Ovule number is also correlated with the deposited pollen loads. Within one species the ovule number has shown a high degree of correlation with the stigmatic area. However, at the genus level and above such a correlation was not observed because of the differences in pollinia reception methods, the consistency of pollinia, and pollen density on the pollinators. Aggregation of pollen into pollinia seems to be an adaptation to the increasing potential seed productivity of orchid flowers. The energetic provision for this intensification in orchids was obtained as a result of the strong economization of the whole reproductive process under the conditions of a decrease of the visitation frequency. This is the main characteristic of the reproductive strategy in the orchids studied.

ORCHIDS HAVE the highest seed production per fruit among Magnoliophyta; fruits of many of the monandrous orchids contain a million or more seeds. A special pollination system is needed to fertilize such a high quantity of ovules at one time. The outstanding contrivances of orchid flowers to pollination by insects are well known (Darwin, 1862; van der Pijl and Dodson, 1966). The most important of these is the pollen aggregation into pollinia. Aggregation of pollen into pollinia has two consequences for the pollination system of the orchids. First, the pollinia as a whole are removed and transported to the stigmas by pollinators in an "all-or-nothing" principle. Second, pollen in pollinia are delivered to the stigmas in larger portions (van der Pijl and Dod-

son, 1966). With regard to the latter, the overall majority of authors discussed the high ovule number in each ovary (Darwin, 1962; van der Pijl and Dodson, 1966; Dressler, 1981, 1983; Benzing, 1987).

The size of pollen load on the stigma in orchids depends on the consistency of pollinia. Hard pollinia are delivered as single units (van der Pijl and Dodson, 1966, Montalvo and Ackerman, 1987, Proctor and Harder, 1994), whereas in species with soft and sectile pollinia only a small part from each pollinarium is delivered onto the stigmas (Darwin, 1862; Gregg, 1991; Proctor and Harder, 1994). Therefore, Proctor and Harder (1994) postulated that "size" of the typical unit of pollen deposition rather than that of the entire pollinarium evolved to match ovule number in orchids." Later Neiland and Wilcock (1995) obtained results which confirmed this logical hypothesis in the case of some European orchids with sectile pollinia. Their pollinia contain much more pollen

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TABLE 1. Systematic position and collection localities of the species studied

Subfamily Epidendroideae	
Tribe Maxillarieae	
Subtribe Stanhopeinae	
<i>Coryanthes senghasiana</i> G. Gerlach	
Tribe Neottieae	
Subtribe Limodorinae	
<i>Cephalanthera damasonium</i> (Mill.) Druce	KRA
<i>C. longifolia</i> (L.) Fritsch	KRA
<i>Listera ovata</i> (L.) R.Br.	KRA
Subfamily Orchidoideae	
Tribe Orchidae	
Subtribe Orchidinae	
<i>Orchis coriophora</i> L.	PER
<i>O. morio</i> (L.)	KRA
<i>O. picta</i> Loisel.	ORE
<i>O. provincialis</i> Balb. Ex DC.	KRA
<i>O. simia</i> Lam.	SCA
<i>O. tridentata</i> Scop..	PER
<i>Comperia comperiana</i> (Steven). Asch. & Graebn.	KRA
<i>Dactylorhiza romana</i> (Sebast.) Soó	KRA
<i>D. incarnata</i> (L.) Soó	SCA
<i>Ophrys mammosa</i> Desf. subsp. <i>mammosa</i>	PER
<i>O. scolopax</i> subsp. <i>cornuta</i> (Stev.) E.G.Camus	KRA
<i>Platanthera bifolia</i> (L.) Rich.	KRA
<i>P. chlorantha</i> (Custer) Rchb.	SCA
<i>Steveniella satyrioides</i> (Steven) Schltr.	

Classification after Dressler (1993) and Buttler (1986)

KRA = Krasnolesie, ORE = Oreanda, PER = Peredovoe, SCA = Scalstoe (Crimea); VAU = The ovary of *Coryanthes senghasiana* was taken from a cultivated plant in a greenhouse of the Botanical Gartenin Munich (Germany). This plant came originally from Colombia, Dept. Vaupés.

than all available ovules in the ovary. The pollen/ovule (P/O) ratios of species with hard pollinia have not been investigated hitherto. Therefore, we examined the influence of the pollinium structure (soft, sectile, and hard) on ovule number in orchids.

MATERIAL AND METHODS

The systematic position and localities of collection of the species studied are enumerated in Table 1. They have one of three types of pollinia: soft (Limodorinae), sectile (Orchidinae), and hard (Stanhopeinae).

The soft pollinia of Limodorinae species were stained in acetocarmine directly on a glass slide for minimum of two hours. Afterwards pollen monads or tetrads of the pollinia were dispersed in one layer by gradually pressing on the cover-slip. Pollen grains were photographed with an MBS-15 microscope using a Heliopan N 11 yel-

low-green light filter. The number of pollen grains on the slide was counted on their photoimages.

Pollinia of Orchidinae are sectile, forming massulae. Massulae are harder than the pollinia of Limodorinae. To count the number of pollen grains in Orchidinae flowers, the hemipollinaria were taken from the opened flowers. Each hemipollinarium was thoroughly immersed with the stigma secretion of the same species (normally it is laid between two stigmas and slightly squeezed). Then the hemipollinarium with the stigmas was put into a separate tube with 0.3 ml of 10% glucose solution. An hour later 1 ml of acetocarmine was added. After several days of staining the hemipollinaria were stored in glycerine. The colored massulae were spread on a glass slide by gradually pressing on the coverslip to disperse the pollen. Further methods were as above. The general number of pollen in an anther was estimated by the average number of pollen grains in one massula X the average number of massulae in a hemipollinarium and then doubled.

The hard pollinia of *Coryanthes* are resistant to mechanical destruction. Therefore, the pollinium first was wholly immersed into stigmatic secretion for five hours. Afterwards it was hydrolyzed in 1 N HCl for 30 minutes and then stained with acetocarmine. The stained pollinium was placed on a glass slide and broken into pieces with a razor blade and then treated as above. The number of pollen grains in the anther of *C. senghasiana* was determined by counting the pollen of one pollinium and then doubled because one pollinarium of these orchids consists of two pollinia.

At the beginning of anthesis the placental tissue in European orchids investigated differentiates only in the ovule primordia. Therefore, the number of ovules in one ovary of these orchids was determined by the number of their primordia. The method of counting the number of ovule primordia of the European orchids has been described earlier and is presented here in a short form (Nazarov, 1989).

After the opening of the flower the ovary is separated from the perianth petals and placed in FAA mixture which contains 70% alcohol, 40% formalin, and glacial acetic acid in the ratio of 10:3:1. In this mixture the ovaries can be stored for maximum one year. After one month the ovaries are transferred from the FAA mixture to the 1N HCl for 5–10 minutes, depending on the car-

TABLE 2. Pollen number per flower in Epidendroideae species.

Taxon	Number of pollinia investigated*	Pollen number per pollinium		Number of theca investigated**	Pollen number per theca		Pollen number per anther
		min-max	M		min-max	M	
<i>Coryanthes senghasiana</i>	1 (1)		400000				800000
<i>Cephalanthera damasonium</i>			5814	8 (3)	6046–19742	11627	23254
<i>C. longifolia</i>			5547	6 (3)	5728–18948	11093	22186
<i>Listera ovata</i>	60 (4)	2464–19040	10791	30 (4)	5668–36864	21583	43166

* The number of hemipollinaria counted are shown in brackets.

** The number of plants counted are shown in brackets.

pel thickness. Then the ovaries are dissected along the carpel sutures and stained for 10–15 minutes in a solution of 1% lightgreen in 70% alcohol. After staining, all placentas with ovule primordia are removed from the ovary with preparation needles and placed in a drop of glycerin. Then the ovule primordia were gradually pressed with the cover glass into one layer and photographed in transmitted light with an MBS-10 binocular microscope. For better contrast an orange light-filter Heliopan N 22 was used. The number of the primordia was counted on their photo-images.

In the majority of tropical orchids the placenta does not differentiate in the ovule primordia during anthesis, so the ovary of *Coryanthes senghasiana* was fixed in an FAA mixture 30 days after pollination. Ovule primordia in the ovary of the pollinated flower were determined as in European orchids.

Length of the ovary was measured with the help of vernier callipers. Stigmatic areas were determined by drawing its contours on millimeter net paper in 1:10 magnification. The extension of the projective area of the hemipollinaria (pollinia) and of the pollen-bearing area on the pollinators was measured and calculated.

RESULTS

The number of pollen grains per flower—All species studied are monandrous orchids, and so the number of pollen grains per flower corresponds to the number of pollen grains per anther. In Limodorinae, all pollen of an anther is aggregated into four pollinia. Each pollinium of *Listera ovata* consists of 616–4,760 pollen tetrads. The number of pollen grains in the thecae of one anther did not differ significantly. Therefore, we de-

termined the pollen number in other Limodorinae species only in one theca. The pollinia of one theca of *Cephalanthera damasonium* consists of 6,046–19,742 pollen monads, and those of *C. longifolia* consist of 5,728–18,948 ones. The mean value and range of pollen monads per soft pollinium of the Limodorinae are shown in Table 2.

In Orchidinae, all the pollen of the anther is aggregated into four pollinia that are sectile with many massulae. Two pollinia from one theca of the anther have their own caudicle and viscidium. This associated structure corresponds functionally with the pollinaria of other orchids; however, only half of the pollen of the anther is aggregated in it. Dressler (1981) called these structures hemipollinaria. One hemipollinarium of the species studied consists of 60–560 massulae on average. Each massula consists of 7 to 869 pollen tetrads. The mean values and ranges of the number of massulae per hemipollinarium, number of pollen grains per massula, as well as the quantity of the studied units for every species are shown in Table 3.

The number of pollen grains per massula changes remarkably even within one hemipollinarium. The apical part of the hemipollinarium contains the largest massulae with the most pollen, and basal massulae are the smallest. *Ophrys* species have the largest massulae and the most pollen grains per massula, especially *O. scolopax* ssp. *cornuta* (Fig. 1). Every apical massula in this orchid contains about 3,000 pollen grains (Table 3). The smallest massulae with the lowest number of pollen were found in *Platanthera bifolia* which also had the smallest difference in the number of pollen between apical and basal massulae (Table 3). *Dactylorhiza incarnata*, too showed few differences between apical and basal massulae (Fig. 1). Other Orchidinae species are intermedi-

TABLE 3. Pollen number per flower in Orchidoideae species.

Taxon	Number of massulae investigated*	Pollen number per massula		Number of hemipollinaria investigated**	Massulae number per hemipollinarium		Pollen number per hemipollinarium	Pollen number per anther
		min–max	M		min–max	M		
<i>Comperia comperiana</i>	80 (3)	88–2593	1063.9	16 (5)	130–177	157.1	167139	334278
<i>Dactylorhiza incarnata</i>	11 (1)	71–723	423.0	24 (6)	87–149	115.1	48687	97374
<i>Dactylorhiza romana</i>	42 (1)	60–996	448.3	24 (8)	80–155	112.2	50299	100598
<i>Ophrys mammosa</i> ssp. <i>mammosa</i>	45 (1)	98–2347	1082.9	10 (3)	75–104	89.9	97353	194706
<i>O. scolopax</i> ssp. <i>cornuta</i>	50 (2)	123–3476	1265.6	8 (20)	91–112	99.1	125421	250842
<i>Orchis coriophora</i>	64 (2)	61–442	223.8	44 (8)	82–175	137.3	30728	61456
<i>O. picta</i>	18 (1)	60–1008	503.1	26 (8)	61–138	95.2	47895	95790
<i>O. provincialis</i>	51 (2)	68–1303	477.8	8 (2)	94–148	123.2	58865	117730
<i>O. simia</i>	97 (3)	42–1004	382.2	10 (3)	97–150	128.4	49074	98148
<i>O. tridentata</i>	10 (1)	73–1068	429.4	13 (3)	64–111	84.1	36113	72226
<i>Platanthera bifolia</i>	165 (3)	32–348	151.6	20 (6)	201–459	358.5	54349	108698
<i>Platanthera chlorantha</i>	138 (3)	28–388	185.0	26 (9)	300–561	436.2	80697	161394
<i>Steveniella satyrioides</i>	30 (2)	76–772	350.5	12 (3)	94–161	126.8	44443	88886

* The number of hemipollinaria counted are shown in brackets.

** The number of plants counted are shown in brackets.

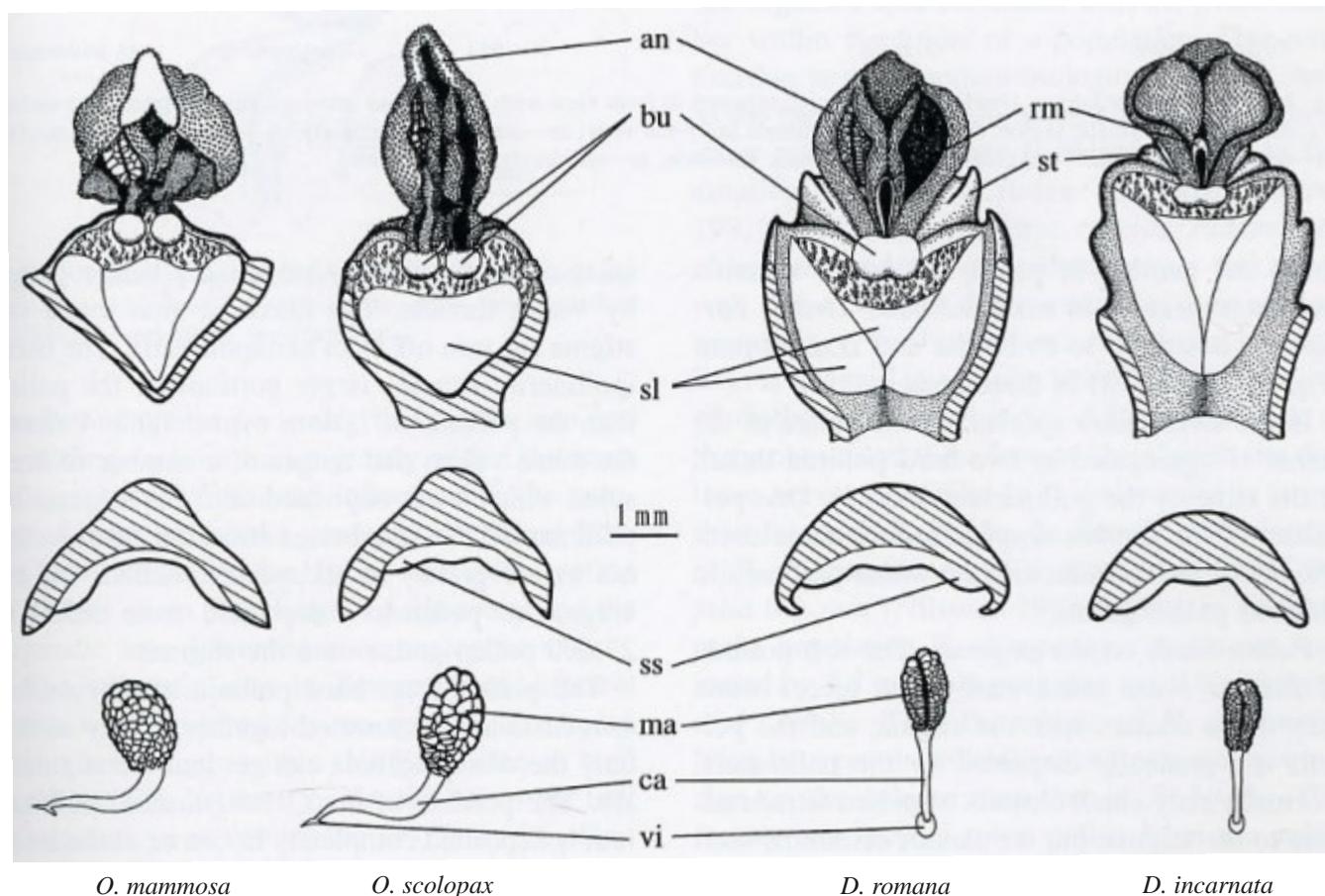


Fig. 1. Floral morphology in *Ophrys* and *Dactylorhiza* species: above, column in front view with lip and spur removed; middle, transverse section of column in the stigmatic region; bottom, hemipollinaria of *Ophrys* species in lateral view and *Dactylorhiza* in frontal view; an—anther, bu—bursicle, ca—caudicle, ma—massulae, rm—rostellum midlobe, sl—sigma lobe, ss—stigmatic secretion, st—staminode, vi—viscidium.

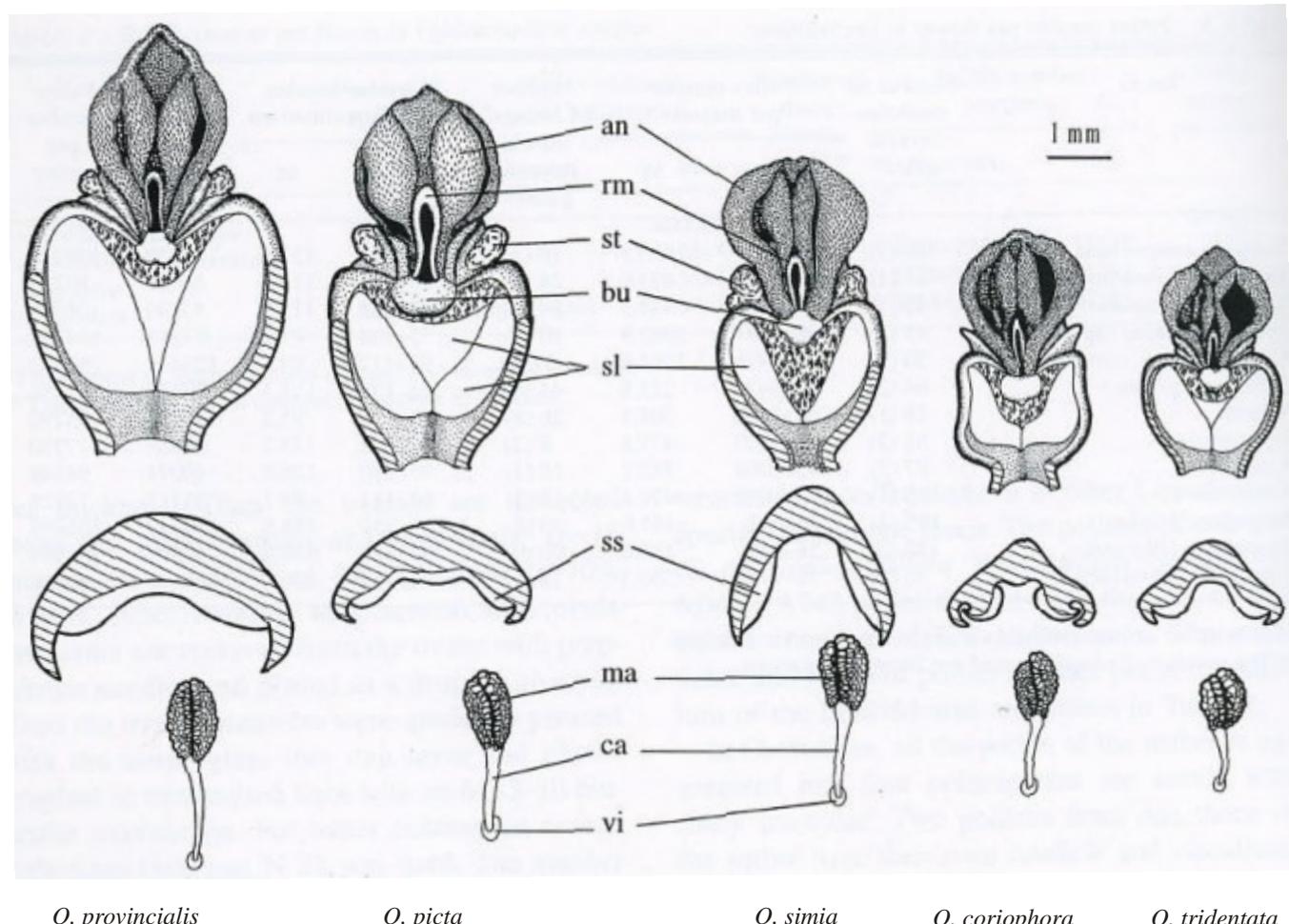


Fig. 2. Floral morphology in *Orchis* species: above, columns in front view with lip and spur removed; middle, transverse section of column in the stigmatic region; bottom, hemipollinaria in frontal view; an—anther, bu—bursicle, ca—caudicle, ma—massulae, rm—rostellum midlobe, sl—stigma lobe, ss—stigmatic secretion, st—staminode, vi—viscidium.

ate in the number of pollen grains per massula and heterogeneity in massulae size. *Orchis coriophora* is similar to *P. bifolia* and *D. incarnata* (Fig. 1, 2; Table 3) in these respects.

In the *Coryanthes* species, all the pollen of the anther is aggregated in two hard pollinia linked to the stipe of the pollinaria (Fig. 3). One pollinium of *Coryanthes senghasiana* contains about 100,000 pollen tetrads, and the whole pollinaria 800,000 pollen grains.

Pollen loads on the stigmas—The soft pollinia of *Listera ovata* break easily onto pieces when they make contact with the stigma, and the pollinia are gradually dispersed by the pollinators. Normally only small clusters of pollen tetrades adhere to the stigma, but we cannot determine their number exactly. The pollen of four pollinia of *L. ovata* is theoretically enough for the pollination of about 30 flowers.

In Orchidinae species the pollinators take one or two hemipollinaria from each flower. The mas-

sulae of hemipollinaria are weakly held together by viscin threads. The massulae that touch the stigma are torn off from hemipollinaria. The hemipollinaria disperse larger portions of the pollen than the pollinia of *Listera ovata*. Table 4 shows the mean values and ranges of a number of massulae which were deposited onto the stigmas by pollinators. The number of massulae on the stigma varied greatly in all species studied. On average, the pollinators deposited from 5,500 to 27,600 pollen grains onto the stigmas.

The pollen from hard pollinia of *Coryanthes senghasiana* is connected together solidly so that only the whole pollinia can get into the stigmatic slit. The pollinia of the *C. senghasiana* pollinaria is deposited completely in one or, at the most, two flowers. Thus, 800,000 or 400,000 pollen grains were delivered to the stigmas.

Ovule number and stigmatic area—Mean values and ranges of ovule primordia number in the ovary for each species are shown in Table 5. The

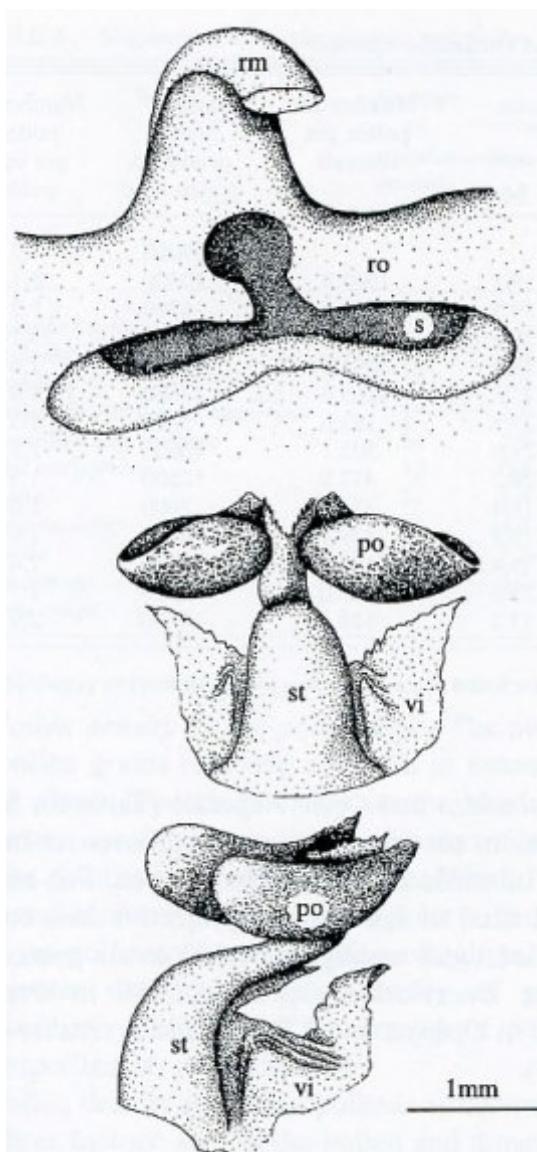


Fig. 3. Rostellum and pollinarium of *Coryanthes senghasiana*; po—pollinarium, s—stigma, st—stipe, rm—rostellum midlobe, ro—rostellum, vi—viscidium.

number of ovules per ovary in Limodorinae and Orchidinae species varies greatly within the same inflorescence. Within one inflorescence the number of ovule primordia gradually decreased from the lower flowers to the higher, with the exception of *Orchis simia*. The length of the ovary and the stigmatic area decreased in an analogous way.

The stigmatic area in flowers from different plants was measured for ten species. The mean values and ranges within this parameter, as well as the relation between ovule number to the unit of stigmatic area, are shown in Table 6. In *Coryanthes senghasiana* the projective stigmatic area was estimated because the stigma is slit-like and the pollinarium is pushed to the depth of the stigmatic channel during pollination.

A regression analysis of the relationships be-

tween ovule number and stigmatic area was carried out only in the four European species. It showed a high degree of correlation at the population level (0.78 for *Orchis provincialis*, 0.83 for *O. picta*, 0.87 for *O. tridentata*, and 0.90 for *Ophrys scolopax* ssp. *cornuta*). Thus the ratio of number of ovules/stigmatic area is more or less constant for each species. However, this ratio differed greatly at the genus level and above (Table 6).

DISCUSSION

Pollen loads on the stigmas—The size of deposited pollen loads in species studied varies greatly. However, the number of pollen deposited per one ovule shows little variation (Table 4). Thus in orchids as well as in other flowering plants ovule number in ovaries is correlated to the pollen number deposited on the stigmas. It is known that the amount of pollen that is delivered to the stigma per pollinator-visit depends first of all on the size of the stigmatic area.

The stigmatic area—In five Orchidinae species, the stigmatic area correlated with the ovule number within the limits of a population. This relationship is an important biological issue, as well as the fact that “the larger stigmas should receive more pollen grains per pollinator visit than the smaller stigmas” (Cruden and Miller-Ward, 1981). The two sympatric orchids *Platanthera chlorantha* and *P. bifolia* demonstrate this thesis well. These species differ mainly in their column morphology. In *Platanthera chlorantha* the column is adapted to viscidia attachment to the eyes of moths, whereas viscidia of *P. bifolia* stick to the proboscis of these insects. As a result the distance between viscidia in *P. chlorantha* is larger than those of *P. bifolia*. In Sweden the viscidia of *P. chlorantha* are 3.4 mm apart, and in *P. bifolia* 0.7 mm (Nilsson, 1983, 1985). In the Crimean population *P. chlorantha* viscidia are separated by 3.5 mm, whereas they are 0.8 mm apart in *P. bifolia*. Thus the stigma of *P. chlorantha* lying between the viscidia occupies a much larger area on the column than that of *P. bifolia*. The former can receive more pollen from the pollinator than the second (Table 4). This may determine the difference in the potential seed productivity (PSP) in these orchids. There are approximately 45% more ovules in an ovary of *P. chlorantha* than in that of *P. bifolia* (Table 5).

TABLE 4. Size of deposited pollen loads in *Coryanthes senghasiana* and Orchidinae species.

Taxon	Number of stigmas investigated	Number of massulae on the stigma*		Number of pollen per massula	Size of typical deposited pollen load	Number of pollen per one ovule
		min–max	M			
<i>Coryanthes senghasiana</i>					800000	1.2
<i>Comperia comperiana</i>	11	3–56	19.1	1063.9	20300	2.1
<i>Dactylorhiza incarnata</i>	18	5–81	39.5	423.0	16700	2.2
<i>Dactylorhiza romana</i>	26	4–104	28.6	448.3	12800	3.1
<i>Ophrys mammosa</i> ssp. <i>mammosa</i>	14	2–81	18.5	1082.9	20000	1.6
<i>O. scolopax</i> ssp. <i>cornuta</i>	25	3–101	21.8	1265.6	27600	1.6
<i>Orchis coriophora</i>	14	3–67	25.1	223.8	5600	2.3
<i>O. picta</i>	24	2–59	21.0	503.1	10600	2.1
<i>O. provincialis</i>	13	2–74	26.2	477.8	12500	1.3
<i>O. simia</i>	14	4–43	18.4	382.2	7000	2.0
<i>O. tridentata</i>	15	8–48	19.5	429.4	8400	1.9
<i>Platanthera bifolia</i>	7	23–50	36.4	151.6	5500	1.4
<i>Platanthera chlorantha</i>	19	16–104	53.6	185.0	9900	1.7
<i>Steveniella satyrioides</i>	15	21–94	57.2	318.3	18200	2.9

* The flowers with whole hemipollinaria deposited on stigma were not included because of the low germination possibility of distal massulae.

At the genus level and above, however, the correlation between the stigmatic area and ovule number are often inverted. For example, there are more ovules in the ovary in *Ophrys scolopax* ssp. *cornuta* and a smaller stigmatic area than in *O. mammosa* ssp. *mammosa*. This can be observed by comparing the *Ophrys* species with *Orchis* *provincialis* and *Coryanthes senghasiana* with

Dactylorhiza and *Ophrys* species (Table 6). Such inversions are caused by strong differences in the ratio of ovule number/stigmatic area. For example, 1 mm² of the stigma's projective area correspond to an average of 1,700 ovule primordia in the *Dactylorhiza* species, 2,300 in *Orchis*, 4,600 in *Ophrys*, and 222,300 in *Coryanthes* (Table 6).

TABLE 5. Ovule number per flower and P/O ratios.

Taxon	Number of investigated ovary*	Ovary length during anthesis	Number ovules in the ovary (PSP of the flower)			P/O ratios	Methods of pollen reception
			min–max	M	±		
Epidendroideae							
<i>Cephalanthera longifolia</i>	16 (7)	11.7	2590–13637	7650	825	2.9	scraping
<i>Cephalanthera damasonium</i>	15 (6)	14.1	4280–11601	7501	606	3.1	scraping
<i>Coryanthes senghasiana</i>	1	54.0		689000		1.2	scraping
<i>Listera ovata</i>	12 (3)	4.0	532–2165	1410	214	30.6	contact
Orchidoideae							
<i>Comperia comperiana</i>	9 (3)	15.6	5352–11739	9231	591	36.2	contact
<i>Dactylorhiza incarnata</i>	37 (8)	11.3	4110–12493	7756	321	12.6	contact
<i>D. romana</i>	35 (11)	12.0	2187–8019	4057	233	24.8	contact
<i>Ophrys mammosa</i> ssp. <i>mammosa</i>	16 (5)	12.7	8718–17154	12566	652	15.5	contact
<i>O. scolopax</i> ssp. <i>cornuta</i>	20 (7)	16.3	7898–33192	17373	1222	14.4	contact
<i>Orchis coriophora</i>	24 (4)	9.4	1383–3840	2410	143	25.5	contact
<i>O. picta</i>	17 (5)	10.8	1320–8460	5123	470	18.7	contact
<i>O. provincialis</i>	30 (8)	16.9	5314–14985	9478	431	12.4	contact
<i>O. simia</i>	34 (5)	9.8	2327–5415	3428	159	28.6	contact
<i>O. tridentata</i>	38 (5)	8.9	2721–8844	4452	179	16.2	contact
<i>Platanthera bifolia</i>	42 (9)	13.2	985–8079	4004	304	27.1	contact
<i>P. chlorantha</i>	31 (10)	17.9	2598–9726	5831	302	27.7	contact
<i>Steveniella satyrioides</i>	28 (5)	7.2	1139–12537	6298	481	14.1	contact

TABLE 6. Stigmatis area, ovule number, and pollen density in pollinia.

Taxon	Number of stigmas investi- gated	Stigmatic area, mm ²			Number of ovules in the ovary	Ovule number per mm ² stigmatis area	Pollen number per mm ² of pollinium's projective area in apical view
		min–max	M	±			
<i>Coryanthes senghasiana</i>	1		3.10		689000	222260	571000
<i>Dactylorhiza incarnata</i>	10	2.4–4.9	3.61	0.21	7756	2150	190000
<i>D. romana</i>	12	2.0–3.8	3.17	0.18	4057	1280	160000
<i>Ophrys mammosa</i> ssp. <i>Mammosa</i>	13	2.8–4.4	3.38	0.16	12566	3720	182000
<i>O. scolopax</i> ssp. <i>cornuta</i>	14	2.9–4.3	3.18	0.14	17373	5460	246000
<i>Orchis coriophora</i>	12	0.8–1.4	1.04	0.14	2410	2320	123000
<i>O. picta</i>	11	1.4–3.1	2.41	0.19	5123	2130	126000
<i>O. provincialis</i>	21	4.7–6.4	5.19	0.09	9478	1830	190000
<i>O. simia</i>	15	1.2–2.7	1.62	0.11	3428	2120	164000
<i>O. tridentata</i>	17	1.2–2.9	1.50	0.10	4452	2970	150000
<i>Listera ovata</i>							77000

Pollen density on the pollinators—The number of pollen grains reaching a stigma in entomophilous plants depends not only on the size of the stigmatic area but also on the density of pollen on the pollinator (Cruden and Miller-Ward, 1981). The orchids have probably the highest pollen density on pollinators among Magnoliophyta. This parameter depends on the density of pollen packed into pollinia and the density of pollinia (hemipollinia) on pollinators.

Pollen density in orchid pollinia is determined by three factors: size of the pollen and dimension and structure of the pollinium. It seems obvious that the pollen density increases exponentially when size of the pollen grain decreases and/or the pollinium dimensions increase. In the species studied, the pollen size decreases, but pollinium dimensions increase in the following sequence: Limodorinae → Orchidinae → Stanhopeinae. Structure of the pollinia also influences pollen density. The pollen in hard pollinia of *Coryanthes senghasiana* is packed more solidly than the pollen in the sectile hemipollinia of Orchidinae. The cumulative influence of these factors explains the highest pollen density in *Coryanthes senghasiana* and the lowest pollen density in *Listera ovata* (Table 6).

The density of pollinia (hemipollinia) on pollinators is also determined by various factors. It depends on the number of pollinia and the pattern of their allocation on pollinators. Obviously pollinia density will decrease with the increase of pollinia number and the uncertainty in the allo-

cation of pollinia on the pollinators. Both factors depend on the level of morphological conformity of the orchid flowers to the pollinators and the frequency of flower visits. For example, pollinators of *Orchis picta* differ considerably in morphology. The number of deposited hemipollinia and the pattern of their allocation on the pollinators differed to the same extent. Each *Eucera longicornis* male transported two hemipollinia. Queens of *Bombus agrorum*, both sexes of *Eucera nigrilabris*, and workers of *Apis mellifera* carry 2.8, 5.1, and 7.0 hemipollinia per individual, respectively. The pollen-bearing area in *Orchis picta* pollinators increased from 1.0 to 6.9 mm². However, the pollen load on the pollinators with many hemipollinia is loosely packed (Fig. 4). Therefore, the pollen density on *Eucera longicornis* (90,800 pollen grains/mm²) is higher than on *Apis mellifera* (80,500), *Eucera nigrilabris* (76,300), and *Bombus agrorum* (35,000).

Ophrys species are pollinated as a rule from one or at the most two different species of males bees in each population. The hemipollinia number and the pattern of their allocation on the pollinators are less variable than in *Orchis picta*. The hemipollinia of *Ophrys scolopax* ssp. *cornuta* occupy a smaller area on the pollinators than *Orchis picta* hemipollinia (Fig. 4). The pollen density on *O. scolopax* ssp. *cornuta* pollinators is remarkably higher in this case (190,600 pollen grains/mm²).

The flower morphology of *Coryanthes* allows only one pollinarium per bee because of the ob-

ligate passage of pollinators from stigma to pollinarium. The position of the pollinaria is always exactly the same: the pollinia lie flat on the pollinators body (Fig. 5). Pollen density on the pollinators is the highest among species studied (457,000 pollen grains/mm²).

Pollinia structure—The pollen load that is delivered to stigmas is not only a function of stigmatic area and pollen density on the pollinator but also the consistency of pollinia. Obviously the deposited pollen load given equal contact area of the pollinia with the stigma and equal pollen density will be larger with hard pollinia than with sectile or soft ones. In Orchidaceae the size of the deposited pollen load increases exponentially with enhanced size of the elementary pollen aggregate.³ The typical deposited pollen load in *Dactylorhiza romana* is up to about two times less than in *Ophrys scolopax* ssp. *cornuta* and 60 times less than in *Coryanthes senghasiana*, although their stigmatic areas are equal (Table 4, 6). The pollen number in elementary pollen aggregates also increased (Table 2, 3).

Size of the elementary pollen aggregate and ovule number—As shown above, in orchids the size of the elementary pollen aggregate is correlated with the amount of pollen deposition on the stigma. Therefore, it is no wonder that the ovule number in the species studied increases in a direct ratio to the increase in size of the elementary pollen aggregate. The highest ovule number per flower is in *Coryanthes senghasiana*, in which the elementary pollen aggregate is the pollinium. It contains half of the pollen of the anther. In Orchidinae, in which the elementary pollen aggregates are the massulae, the ovule number is 40–290 times less than in *C. senghasiana*. *Listera ovata*, in which the elementary pollen aggregate is tetrads, has about 2–12 times fewer ovules than Orchidinae species (Table 5). These data confirm Proctor and Harder's (1994) hypothesis that size of the elementary pollen aggregate (the typical unit of pollen deposition) rather than that of the entire pollinarium determines ovule number in orchid flowers.

Adaptations of the stigmas to reception of ag-

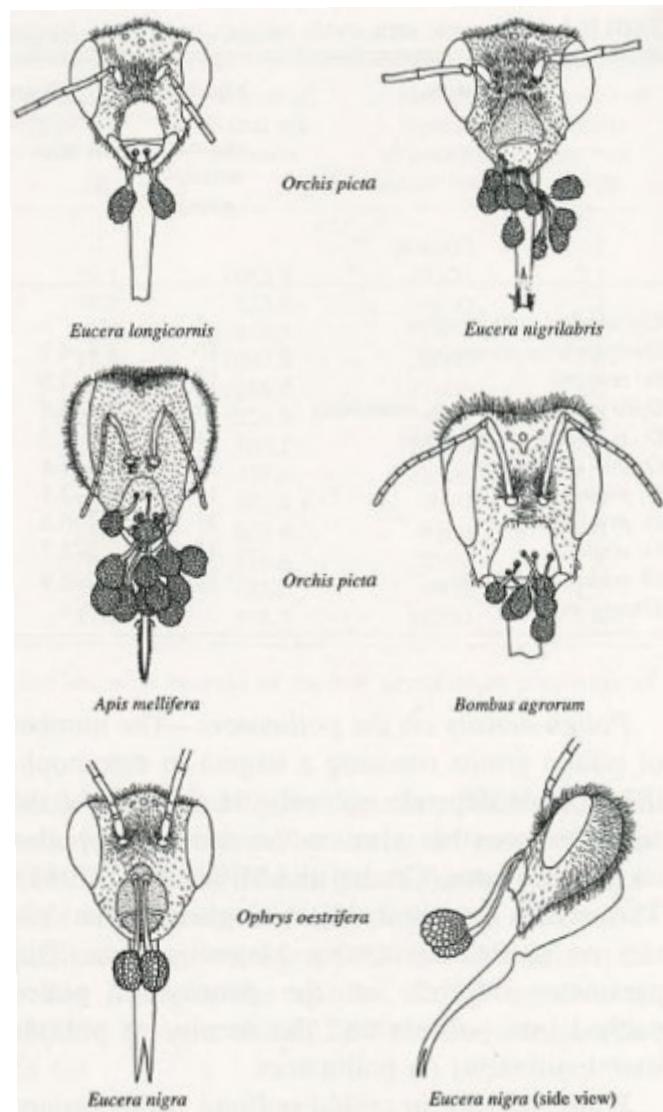


Fig. 4. Position and number of pollinaria on the heads of selected pollinators.

gregated pollen—Large pollen masses such as massulae and pollinia cannot be effectively received by the stigma types present in other plants. Orchid stigmas have a special adaptation for this purpose—a sticky secretion. Darwin (1862) showed the importance of the stickiness of the stigma for the reception of the massulae in Orchidinae. Massulae adhere to the stigma more strongly than they are held together by their viscin threads.

Second, the stigmatic secretion in orchids is thick so that allow pollen aggregation are deeply immersed. In Orchidinae the thickness of the sticky secretion is approximately equal to the size of the massulae (Fig. 1, 2). However, in many species the massulae often do not go deeper into the secretion than up to a third. The level of their immersion perhaps depends on their position in

³ Under ‘elementary pollen aggregates’ we understand such pollen aggregates which are not separated during pollination. Correspondingly *Listera ovata* have tetrades, the *Orchidinae* species—massulae and *Coryanthes senghasiana*—whole pollinia as elementary pollen aggregates.

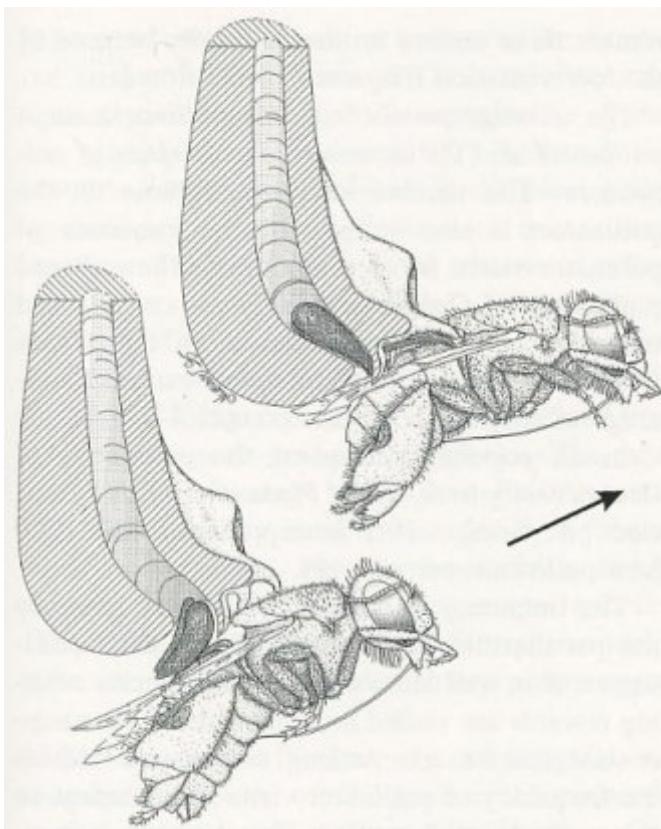


Fig. 5. Pollination mechanism of *Coryanthes* species; rostel-lum scraping pollinia from the pollinator.

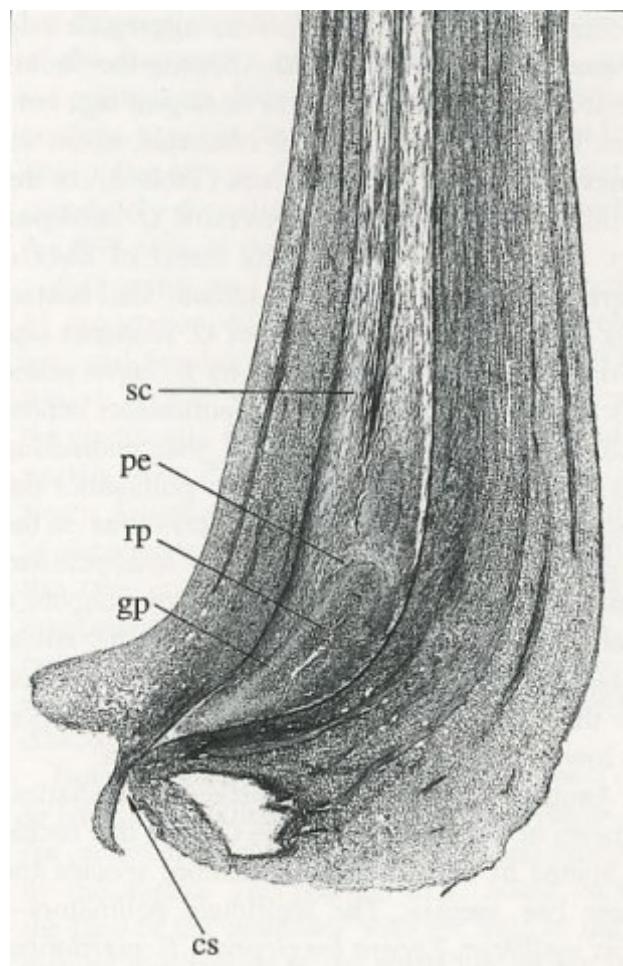


Fig. 6. Column section (longitudinal) of a pollinated *Cory-anthes speciosa* flower; sc—stigmatic channel; pe—pollinium, exine; rp—resting pollen; gp—germinated pollen, cs—closed stigma.

the pollinia, consistency of the secretion, and the shape of the secretory surface of the stigma.

Third, the shape of the secretory surface of the stigma is an important condition for the effective reception of the aggregated pollen. It is obvious that the stigmas with a flat secretory surface are in contact with only a small part of a round pollinium. The secretory surface of some other Orchidinae species deepens around the stigmatic canal and have a "V"-shaped from in transversal section (Fig. 1, 2). The receptive capacity of the "V"-shaped stigmas of *Orchis simia*, *O. coriophora*, and *O. tridentata* (measuring by the ovule number/mm²) is higher than in relatively flat stigmas of *Orchis provincialis* and *Dactylorhiza romana*, although they have smaller massulae than *O. provincialis* and *D. romana* (Table 3, 6).

Shape of the secretory surface of Orchidinae stigmas is correlated with the morphology of hemipollinaria. In species with a curved stigma the inner parts of the hemipollinarium have possibly much less contact with the stigma than the outer ones. This would explain the differences in size and number of massulae between the outer and inner parts of hemipollinaria. These differ-

ences can be seen particularly well in species with a sterile stigma midlobe (Fig. 2).

The hard pollinia of *Coryanthes senghasiana* considerably exceed the massulae of Orchidinae in size. The production of large pollen masses inevitably must correspond to the presence of very deep stigmas. Indeed the stigma of *C. senghasiana* has the form of a deep gullet, filled with secretion (Fig. 3). Pollinia of *C. senghasiana* recede into the stigmatic slit so that only their tips protrude after being deposited by the pollinator (Fig. 5). After pollination the edges of stigma begin to swell and pull the pollinia inside the stigmatic channel. The following day the stigmatic slit is closed, and the pollinia are submerged completely in the secretion (Fig. 6). Obviously, effective germination of the pollen in this orchid would not be possible without this special mechanism of actively pushing the pollinia into the stigmatic cavity.

Size of the elementary pollen aggregate and frequency of pollinator visits—Among the Orchidinae species studied *Ophrys scolopax* ssp. *cornuta* bears the largest apical massulae, about 28 times larger than the basal ones (Table 3). In the Crimean populations the flowers of *O. scolopax* ssp. *cornuta* are pollinated by males of *Eucera nigra* Lep. Males of this bee seldom visit nectarless *Ophrys* flowers. Analysis of *O. scolopax* ssp. *cornuta* hemipollinaria carried by *E. nigra* males has shown that only 5% of the pollinations deposited all massulae on the stigmas, thus pollinating the flowers. The majority of the pollinators deposited less than one third of the massulae on the stigma from the apical part of the hemipollinarium (Nazarov, Ivanov, and Holodov, unpubl.). Here the significant enlargement of the apical massulae needs to be considered as an adaptation for the increased effectiveness of pollen transport to low visitation frequency of pollinators.

Another situation was observed in nectarless flowers of *Orchis picta*. In the Crimea this orchid is visited by six different bumblebee species and three bee species. The legitimate pollinators—*Apis mellifera*, *Eucera longicornis*, *E. nigrilabris*, and *Bombus agrorum*—visited the flowers more often than the pollinators of *Ophrys*. The dispersal level of massulae by pollinators during pollination was also higher than for *Ophrys scolopax* ssp. *cornuta*. Many *Orchis picta* hemipollinaria on the pollinators captured had less than half of the massulae or only the caudicle. Therefore, the enlargement of apical massulae is weaker in *O. picta* than in *O. scolopax* ssp. *cornuta* (Fig. 1, 2).

The weakest differences between apical and basal massulae were found in *Orchis coriophora* and *Platanthera* species. Apical massulae were only about 7–14 times larger than basal ones (Table 3). These orchid species reward their pollinators with nectar, so their flowers are intensely visited throughout the flowering period (Dafni and Ivry, 1979; Nilsson, 1983), and the likelihood of deposition of basal massulae on the stigmas is higher than in nectarless orchids.

The level of massulae differentiation in the hemipollinarium is linked with the frequency of pollinator visits. Deposition of apical massulae on the stigmas of nectarless orchids is much more probable than basal ones. Basal massulae mostly

remain there unused on the pollinator because of the low visitation frequency of the flower.

The avarage number of hemipollinaria an a pollinator and the attraction peculiarities of pollinators—The number of hemipollinaria on the pollinators is also linked with the frequency of pollinator visits. More than 64% of the captured pollinators of *Ophrys scolopax* ssp. *cornuta* had only one or two hemipollinaria on their frons (Fig. 4). Pollinators of *Orchis picta* carried an average of 6.3 hemipollinaria (range: 2–11). In the Crimean populations studied, the pollinators of *Orchis coriophora* and of *Platanthera bifolia* carried on avarage 10.2 hemipollinaria and 12.0 hemipollinaria, respectively.

The frequency of pollinator visits are linked to the peculiarities of the attraction of their pollinators. It is well known that orchid species offering rewards are visited more intensively than non-reward species are. Among non-reward orchids the frequency of pollinator visits is dependent on the methods of deception. For example, non-reward *Ophrys* are pollinated by certain male species of Hymenoptera trying to copulate with the flowers (pseudocopulation) (Kullenberg, 1961; Kullenberg and Bergström, 1976; Paulus and Gack, 1990a, b). Despite a striking similarity between *Ophrys* flowers and females of their pollinators, the males easily distinguish the deception when females appear in the biotope. This is confirmed by the peculiarities of the pollination syndrome of *Ophrys* species: the beginning of the anthesis coincides with the males leaving their nest; pollination occurs mainly in the first third of anthesis when there are no females in the biotope. The majority of the later flowers remain unpolinated, and fruits develop mainly in the lower parts of the inflorescence (Faegri and van der Pijl, 1979). A similar situation is observed in *Ophrys scolopax* ssp. *cornuta* in the Crimea (Nazarov, Ivanov, and Holodov, unpubl.).

The flowers of *Orchis picta* in the Crimea imitate the flower color or form of several nectariferous plant species, as *Muscari* sp. (Liliaceae), *Ajuga genevensis* L., and *Lamium maculata* L. (Lamiaceae). This similarity is not perfect, but the pollinators search for the nectariferous flowers so persistently that they often visit the nectarless flowers of *O. picta*. Fruit set is about 40 % (Nazarov and Alekseev, unpubl.).

P/O ratios and the size of pollen aggregate—

The ratio of the amount of pollen to the number of ovules in the flower (P/O) is an important quantitative character of the reproductive system in angiosperms. Cruden (1977) believed that "P/O's reflect the likelihood of sufficient pollen grain reaching each stigma to result in maximum seed set. The more efficient the transfer of pollen, the lower the P/O should be." Indeed, he reviewed P/O ratios in 96 plant species and found that ratios were correlated ($r = 0.947$) with the system of sexual reproduction and increased from cleistogamous to xenogamous species. The mean P/O ratio on average was 4.7 in cleistogamous, 27.7 in obligately autogamous, 168.5 in facultatively authogamous, 796.6 in facultatively xenogamous, and 5,859.2 in xenogamous species. These values of P/O were found in species which have the monads as the elementary pollen aggregate.

However, some facultatively and obligately xenogamous species of Onagraceae, Mimosaceae, and Asclepiadaceae did not correspond to Cruden's rule. The low P/O ratios in Onagraceae (407 on average), Mimosaceae (559), and Asclepiadaceae (7) were considered as the evolutionary response to an increase of the efficiency of pollination by aggregated pollen. Pollen of these species are held in clumps by viscin threads in Onagraceae, occur as polyades in Mimosaceae or are united in pollinia in Asclepiadaceae (Cruden, 1977; Cruden and Jensen, 1979). Thus, the species of Onagraceae, Mimosaceae, and Asclepiadaceae show lower P/O ratios with increasing size of the pollen aggregate.

Orchids lie outside Cruden's rule, too. The P/O ratios, which were determined early in some xenogamous orchid species, varied from 3.9 to 24.0 (Mehrhoff, 1983; Neiland and Wilcock, 1995). Among the species studied only *Cephalanthera damasonium* has an authogamous breeding system. The other species are facultatively xenogamous or obligately xenogamous. Their P/O ratios vary from 1.2 to 36.4 (Table 5). Differences in P/O ratios in our studies were not determined by the efficiency of removal and transport of the pollen aggregates. For example, *Listera ovata* has a considerably higher P/O than *Coryanthes senghasiana* (Table 5), although all pollen from their flowers were removed by the first pollinator. The differences in P/O ratios among the species studied are obviously conditioned by the character of pol-

len dispersal, which depends first of all on the size of the elementary pollen aggregate. In *L. ovata* the pollinia are dispersed by pollinators in smaller portions than the hemipollinarium of Orchidinae. The pollen of one *C. senghasiana* pollinaria is completely deposited during one visit. Therefore, the P/O ratio of this orchid is 1.0.

P/O ratios and methods of pollinia reception by the stigma—Studies of Limodorinae, Orchidinae, and Stanhopeinae species show that P/O ratios in orchids decrease with increasing size of the elementary pollen aggregate. However, an unusually low P/O ratio was determined in two North American orchid species with soft pollinia. In facultatively xenogamous *Isotria verticillata* this ratio is 3.9, and in facultative autogamous *I. medeoloides* it is 3.8 (Mehrhoff, 1983). Low P/O ratios (Table 5) were found for xenogamous *Cephalanthera longifolia* (3.0) and autogamous *C. damasonium* (3.1).

Both *Cephalanthera longifolia* and *Listera ovata* have soft pollinia. However, these orchids differ remarkably in the method of pollen reception by the stigma. The pollinia of *L. ovata* stick to the face of the pollinator with the help of the explosive mechanism of the rostellum. When it repeats its visit, the pollinator presses the pollinia on the stigma (Nilsson, 1981). The apical part of the pollinia has the most chance to contact the stigma, and in this case we speak of a "contact" method of pollen reception.

In *Cephalanthera longifolia* the rostellum plays the main role in pollen reception. The pollinia of *C. longifolia* are deposited on the back of the thorax of pollinators (*Halictus* sp.). When the insect visits another flower the rostellum scrapes the pollinia from the pollinators; this is the "scraping" method of pollen reception. With the scraping method a larger amount of pollen is deposited on the stigma. Therefore the P/O ratios in *Cephalanthera* are considerably lower than those in *Listera ovata*, although both species have soft pollinia. The comparison between *Listera ovata* and *C. longifolia* shows that in species with an identical type of pollen aggregation, the P/O can be lower when the pollen is received by the scraping method. However, in each method of pollen reception (e.g. contact or scraping method) the P/O ratio depends on the type of pollen aggregation.

Coryanthes senghasiana also shows the scrap-

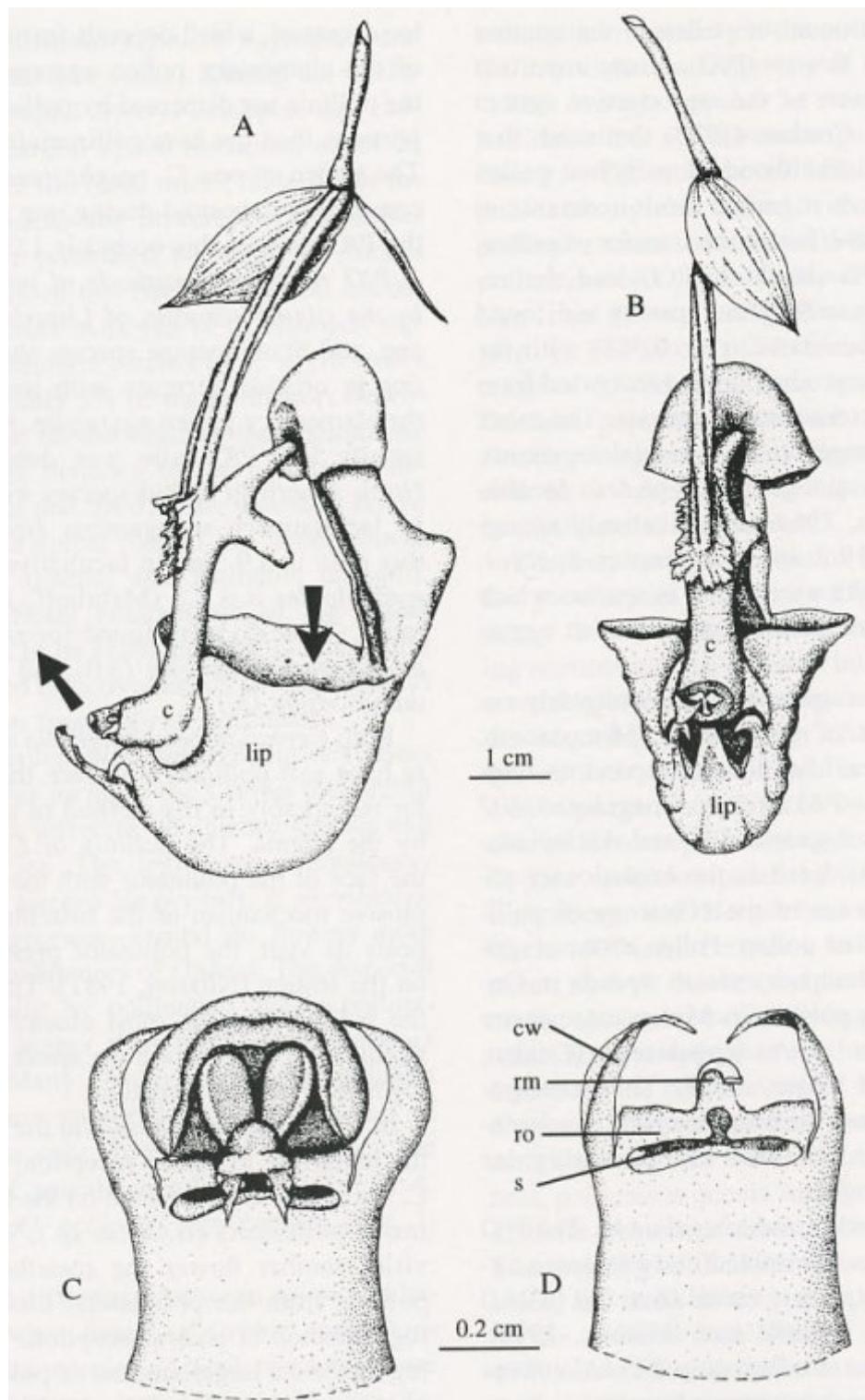


Fig. 7. Views of floral morphology of *Coryanthes senghasiana*. A. Flower in lateral view with sepals, petals and one column wing removed; arrows indicate the path of the pollinator. B. Flower in dorsal view with sepals, petals and one column wing removed. C. Column apex (viewed from above) before the first visit. D. Column apex (like C.) But after the first visit, with pollinarium removed. C—column, cw—column wing, rm—rostellum miglobe, ro—rostellum, s—stigma.

ing method of pollen reception. Its pollination mechanism is similar to that of the trap flowers of Cypripedioideae. The pollinator of a *Coryanthes* species falls in a liquid-filled bucket and can only escape by passing through a narrow tunnel formed by lip and column (Fig. 7). The size of

the pollinating bee and the passage are similar; an exact positioning of the pollinarium is the result. If a pollinarium-carrying bee passes the flower, the pollinia are scraped off by the rostellum (Fig. 5). In *C. senghasiana* the scraping method of pollen reception is combined with the hard pollinia,

and the pollinia are left as a whole on the stigma. This explains a lower P/O in *C. senghasiana* than in *Cephalanthera longifolia*.

The Orchidinae species have a contacting method of pollen reception by stigma. In these orchids only the mouth parts of the insect get inside the flower (Nilsson, 1980, 1983). In this case the insect presses only the apical part of the hemipollinaria into the stigma. These orchids have larger elementary pollen aggregates and lower P/O ratios than *Listera ovata*.

Deceptive pollination systems and ovule number—Orchids have a reputation of being “excellent deceivers.” Approximately one third or more of all orchid species deceive their pollinators (Ackerman, 1986). All means of deceptive attraction are known in orchids. Furthermore, non-traditional means of reward of the pollinators by oil or perfume is widespread in orchids (Faegri and van der Pijl, 1979). Thus, about half of the species of the orchid family do not use nectar as a means for rewarding pollinators. The use of pollen as reward is unavailable because of its aggregation into pollinia, so the phenomenon of nectarlessness of flowers is obviously not casual. The evolution to nectarless flowers took place in different unrelated taxonomic groups of the orchids (Stoutamire, 1983; Dafni, 1984; Ackerman, 1986). Flowers of non-reward species are visited infrequently, and their fruit set was 3–10 times less than in nectariferous species (Dafni and Ivry, 1981a, b; Nilsson, 1980; Ackerman, 1986, 1989; Rodríguez-Robles et al., 1992).

The absence of nectar in orchid flowers has attracted the attention of many authors and has been repeatedly discussed (Darwin, 1862; Dafni, 1984, 1987; Ackerman, 1986). The first one who found a logical explanation for the contradiction between wide distribution of the deceptive pollination systems in orchids and the strong decreasing fruit set in non-reward species was Ackerman (1986), who stated that: “deceptive pollination evolved from reward pollination systems without the loss of reproductive success.” This statement is supported by our result from studies of ovule number in reward and rewardless orchids. Among Orchidinae the ovule number in the ovary increases from nectariferous species to deceptive species (Table 5). The rewardless species in the Crimea produced as many seeds per inflorescence during one vegetative period as nectariferous spe-

cies (Nazarov, 1995), so decreasing fruit set is neutralized by the increasing number of seeds per capsule during the evolution toward deceptive attraction methods.

The reasons for the wide distribution of nectarless orchid flowers is an important issue for the understanding of the evolution of their potential seed productivity (PSP). Ackerman (1986) considered that the predation of fruits and abortion of pollinated flowers in nutrient-limited plants or in self-incompatible species by geitonogamy “may neutralize a high frequency of pollinator visits.” Indeed in the Crimea the two autogamous orchids, *Limodorum abortivum* and *Cephalanthera damasonium*, are preyed upon by caterpillars of *Lobesia crimea* Flkv. (Lepidoptera, Torticidae) and flies of *Chyliza vittata* Meig. (Diptera, Psilidae) more intensively than nectarless species. In some years both predators can damage more than 70% of the ovaries and immature capsules of *Limodorum abortivum*. Fruits of xenogamous orchids are preyed upon only by caterpillars of *L. crimea* and not so intensively. Among them, the nectarless species are preyed upon relatively seldomly and only in particular communities (Nazarov, 1987, 1995).

The influence of ovule differentiation on ovule number—While switching to the deceptive pollination system, orchids economized their plant vigor at the expense of a decreasing nectar production and aborted and damaged fruit. However, this economy could only have been profitable when it compensated for the energy wasted in the production of unpollinated flowers. It is characteristic for orchids to use minimal energy to build up single flowers. In *Tipularia discolor* the cost of an inflorescence is about equal to the cost of a few fruits (Snow and Whigham, 1989). Such a low value was first of all a consequence of the weak differentiation of ovules in the ovary of mature flowers. On the placenta of European orchids, only the ovule primordia are differentiated during anthesis. In the majority of the species studied ovule primordia look like a filamentous row covered by epidermic cells. Only in *Listera ovata* and *Orchis coriophora* are the ovule primordia differentiated into archesporial cells and primordial integuments. The level of ovule differentiation is closely related to the ovule number in the ovary. The number of ovule primordia per mg of phytomass of the column increased in the following

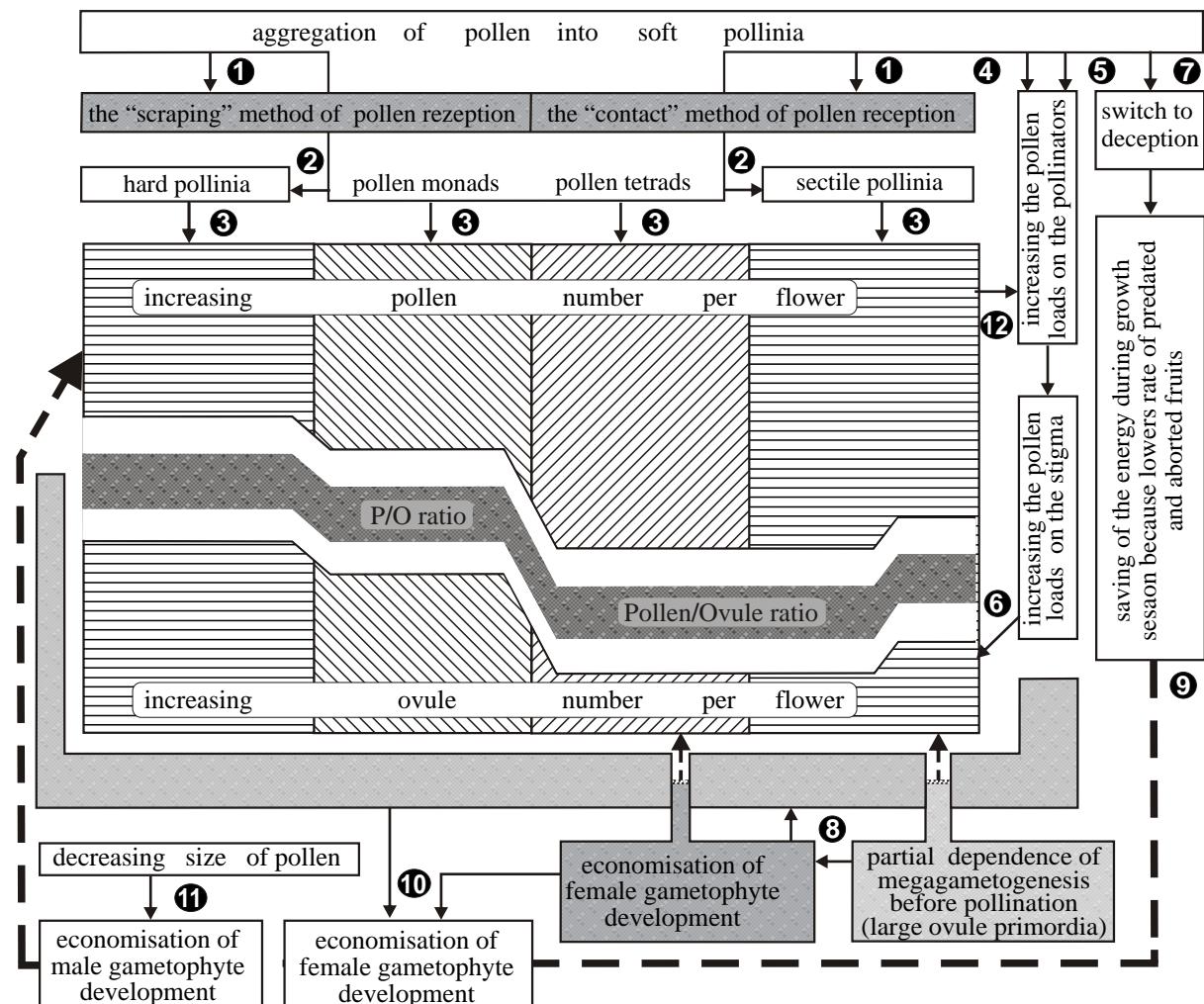


Fig. 8. Schematic representation of the inter-relationships of factors controlling ovule number in orchids.

order: *Listera* (400 per mg) → *Platanthera* (1,700) → *Cephalanthera* (2,000) → *Dactylochila* (2,100) → *Orchis* (2,500) → *Ophrys* (3,100). Ovule number per flower increased accordingly.

Placentae of *Coryanthes senghasiana* are not differentiated in the ovule primordia during anthesis. Therefore, the cost of ovules is excluded from the expenses on the production of unpollinated flowers. Ovules with integumental primordia, funiculus, and archesporial cell are formed 30 days after pollination and mature seed after 70 days. It is obvious that a total depression of ovules before pollination could develop only under the conditions of an uninterrupted or long growing period. In orchids of the temperate zone, the depression of female function of the flower is probably limited by the short growing season (Proctor and Harder, 1994).

Evolution scenario of P/O ratios and increase in seed production in orchids—Our result and

the study of literature mentioned earlier show that potential seed productivity and P/O ratio of orchid flowers are reflected in the fundamental correlations among the plants and their pollinators. The most meaningful correlations are presented in a scheme here.

The rostellum differentiated parallel to the aggregation of pollen into soft pollinia. Rostellar morphology determined the methods of pollinia reception by the stigma (see Figures 8, 1). The method of pollen reception by the stigma has a strong influence on pollinia types. By the "scraping" method, soft pollinia were transformed into hard pollinia and by "contact" methods into sectile pollinia (2). The methods of pollen reception and the types of pollinia determined the levels of P/O ratios in the orchids (3).

Pollen aggregation into pollinia strongly increased the size and density of transported pollen load on the pollinators and in consequence also the deposited pollen load on the stigmas (4). The

aggregation of pollen into pollinia and the forming of an associated structure for pollinia deposition on the pollinators made pollen entirely unavailable for food reward as an attraction. A more economic use of produced pollen facilitated an increase in pollen load, too (5). Ovule number was adjusted to the pollen number deposited on the stigmas (6).

The efficiency of one pollinator visit strongly increased as a result of removing and transporting of pollinia in an "all-or-nothing" principle. This facilitated the switch to deceptive attraction methods (7). Non-reward orchids economized on energy during development of flowers because of the depression of megagametogenesis and megasporogenesis before pollination (8).

The reproductive success in many rewardless orchids is not limited by resource constraints during one year. They saved energy due to the absence of nectar production, less fruit predation, and a low abortion rate of pollinated flowers. The energy cannot be invested, however, in the production of ovules during flowering in the majority of tropical species because of the total depression of megasporogenesis before pollination takes place. The energy flux seems to be completely directed toward pollen production (9). The same is true also for terrestrial orchids of the temperate zone in which megasporogenesis is depressed at the earliest periods of ovule development. Only a small portion of energy flux is invested in ovule development in these orchids. Because of the depression in the development of the female gametophyte during anthesis in orchids, a strong redistribution of energy between female and male reproduction occurs (10). Pollen production per flower can be increased by a reduction in the size of a pollen grain (11), and the increase in pollen number has facilitated an increase in ovule number through higher pollen flux (12).

As the scheme shows, the aggregation of pollen into pollinia seems to be the crossroads for the increasing number of ovules in the ovary. However, the final answer to the question "Have pollinia facilitated the increasing ovule number or has the increasing ovule number facilitated the pollen packaging into pollinia?" is difficult because the two parameters represent one more example of coevolution in orchids. They influence each other in many direct and indirect relationships within other parameters of the pollination

system. Few and scattered investigations on the reproduction in orchids so far do not allow us to gain an exact knowledge of the evolution of the reproductive systems in this family.

LITERATURE CITED

- Ackerman, J. D. 1983. On the evidence for a primitively epiphytic habit in orchids. *Syst. Bot.* 8: 474–477.
- . 1986. Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* 1: 108–113.
- . 1989. Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). *Syst. Bot.* 14: 101–109.
- Benzing, D. H. 1987. Major patterns and processes in orchid evolution: a critical synthesis. Pages 33–77 in J. Arditti (ed.), *Orchid Biology: Reviews and Perspectives, IV*. Cornell University Press, Ithaca and London.
- Buttler, K. P. 1986. *Orchideen*. Mosaik Verlag, München, Germany.
- Cruden, R. W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31: 32–46.
- . and K. G. Jensen. 1979. Viscin threads, pollination efficiency and low pollen-ovule ratios. *Amer. J. Bot.* 66: 875–879.
- . and S. Miller-Ward. 1981. Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: a hypothesis. *Evolution* 35: 964–974.
- Dafni, A. 1984. Mimicry and deception in pollination. *Ann. Rev. Ecol. Syst.* 15: 259–278.
- . 1987. Pollination in *Orchis* and related genera: evolution from reward to deception. Page 79–104 in J. Arditti (ed.) *Orchid Biology: Reviews and Perspectives, IV*. Cornell University Press, Ithaca and London.
- . and Y. Ivri. 1979. Pollination ecology of and hybridization between, *Orchis coriophora* L. And *O. collina* Sol. Ex Russ. (Orchidaceae) in Israel. *New Phytol.* 83: 181–187.
- . and —. 1981a. The flower biology of *Cephalanthera longifolia* (Orchidaceae) — pollen imitation and facultative floral mimicry. *Pl. Syst. Evol.* 137: 229–240.
- . and —. 1981b. Floral mimicry between *Orchis israelitica* Baumann and Dafni (Orchidaceae) and *Bellevallia flexuosa* Boiss. (Liliaceae). *Oecologia (Berlin)* 49: 229–232.
- Darwin, C. 1862. *The Various Contrivances by Which British and Foreign Orchids are Fertilised by Insects, and on the Good Effects of Intercrossing*. J. Murray, London.
- Dressler, R.L. 1981. *The Orchids: Natural History and Classification*. Harvard University Press, London.
- . 1993. *Phylogeny and Classification of the Orchid Family*. Dioscorides Press, Portland, Oregon.
- Faegri, K. And L. Van der Pijl. 1979. *The Principles of Pollination Ecology*. Pergamon Press, Oxford.
- Gregg, K. B. 1991. Defrauding the deceitful orchid: pollen collection by pollinators of *Cleistes divaricata* and *C. bifaria*. *Lindleyana* 6: 214–220.
- Kullenberg, B. 1961. Studies in *Ophrys* pollination. *Zool. Bidrag. (Uppsala)* 34: 1–340.
- . and G. Bergström. 1976. Hymenoptera Aculeata males as pollinators of *Ophrys* orchids. *Zool. Scr.* 5: 13–23.
- Mehrhoff, L. A. 1983. Pollination in the genus *Isotria* (Orchidaceae). *Amer. J. Bot.* 70: 1444–1453.
- Montalvo, A. M. and J. D. Ackerman. 1987. Limitations to fruit production in *Ionopsis utricularioides* (Orchidaceae). *Biotropica* 19: 24–31.

- Nazarov, V. V. 1987. On the damage of the fruits of orchids by the larvae of tortricid moth *Lobesia crimea* Flkv. (Lepidoptera, Tortricidae) in the Crimea (in Russian). *Revue d'Entomologie de l'URSS.* 76: 519–520.
- . 1989. Small seed and ovule calculation technique with special reference to the Orchidaceae family (in Russian). *Bot. J.* 74: 1194–1196.
- . 1995. *Reproductive Biology of Crimean Orchids* (in Russian). Ph.D. Thesis. Komarov Botanical Institute, St. Petersburg.
- Neiland, M. R. M. and C. C. Wilcock. 1995. Maximisation of reproductive success by European Orchidaceae under conditions of infrequent pollination. *Protoplasma* 187: 39–48.
- Nilsson, L. A. 1980. The pollination ecology of *Dactylorhiza sambucina* (Orchidaceae). *Bot. Notiser.* 133: 367–385.
- . 1981. The pollination ecology of *Listera ovata* (Orchidaceae). *Nord. J. Bot.* 1: 461–480.
- . 1983. Processes of isolation and introgressive interplay between *Platanthera bifolia* (L.) Rich. and *P. chlorantha* (Custer) Reichb. (Orchidaceae). *Bot. J. Linn. Soc.* 87: 325–350.
- . 1985. Characteristics and distribution of intermediates between *Platanthera bifolia* and *P. chlorantha* (Orchidaceae) in the Nordic countries. *Nord. J. Bot.* 5: 407–419.
- Paulus, H. F. and C. Gack. 1990a. Pollinators as prepollinating isolation factors: evolution and speciation in *Ophrys* (Orchidaceae). *Israel J. Bot.* 39: 43–79.
- and —. 1990b. Pollination of *Ophrys* (Orchidaceae) in Cyprus. *Pl. Syst. Evol.* 169: 177–207.
- van der Pijl, L. and C. H. Dodson. 1966. *Orchid Flowers: Their Pollination and Evolution*. University of Miami Press, Coral Gables, Florida.
- Proctor, H. C. and L. D. Harder. 1994. Pollen load, capsule weight, and seed production in three orchid species. *Can. J. Bot.* 72: 249–255.
- Rodríguez-Robles, J. A., E. J. Meléndez, and J. D. Ackerman. 1992. Effects of display size, flowering phenology, and nectar availability on effective visitation frequency in *Comparettia falcata* (Orchidaceae). *Amer. J. Bot.* 79: 1009–1017.
- Snow, A. A. and D. F. Whigham. 1989. Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). *Ecology* 70: 1286–1293.
- Stoutamire, W. P. 1983. Wasp-pollinated species of *Caladenia* in South-western Australia. *Austral. J. Bot.* 31: 383–394.