than 2 n. However, there is an interesting exception with one female associated with two males, which produces the largest number of hybrid offspring in both directions of cross (i.e., 294 and 48).

Table 2 shows the between-isofemale line variability. Some *D. mauritiana* lines can produce double the number of hybrids relative to others. The between-line variability implies that the founder females differ [12]. Nevertheless, the average number of hybrid progeny is in the same order of magnitude (65 hybrids) as that yielded by the *D. mauritiana* strain (74 hybrids) at the same density (see table 1).

A strong asymmetry is consistently observed irrespective of the isofemale line tested (Chi-square test, $p < 0.01$). There is a normal sex ratio in the hybrid progeny from the cross between *D. simulans* females and *D. mauritiana* males. By contrast, there is a noticeable, although non-significant, deficiency of males (Chi-square test, $p < 0.05$) in the reciprocal cross between *D. mauritiana* females and *D. simulans* males. This is true of the results shown in both table 1 and table 2.

The success of the hybridization (t) of the cross of *D. simulans* females from the Seychelles with *D. mauritiana* males confirms earlier results [7], irrespective of the density of flies and male/female proportion (tables 1 and 2). By contrast, a noticeable deviation is observed in the reciprocal cross, that is between *D. mauritiana* females and *D. simulans* males from the Seychelles. Our results are only partially consistent with a previous report [7]. We obtained hybrids in 53 % of the crosses involving *D. mauritiana* strains and 47 % of those using *D. mauritiana* isofemale lines, whereas earlier hybridization experiments completely failed to produce hybrids. Nevertheless, when successful, the cross led to a highly variable number of offspring. All the hybrid males dissected (30 per category of cross) showed normal, although aspermic, gonads but immature cysts were observed.

In our study, the success of the reciprocal cross (t) increases with an increase in the number of flies but not in the male/female proportion (table 1). Many workers have concluded that density influences mating success but for different, sometimes conflicting [13 - 16] reasons. Our results show that the increasing proportion of males does not affect the mating success between *D. mauritiana* and *D. simulans*, whereas it was shown to increase that between *D. melanogaster* and *D. simulans* [11]. Similarly, it was reported [16] that the mating success of *vestigial* males is enhanced by the presence of winged (*ebony*) males, suggesting that the behavior of females may be shifted by the presence of more than one male.

In the Hawaiian lek species, *D. grimshawi*, the frequency of courtship displays was linearly dependent upon male density but agonistic and communal displays were density-dependent only when females were present [17]. However, it was also argued that when males outnumbered females this factor significantly slowed mating [16]. Otherwise, if larval overcrowding could account for the discrepancy between the increasing number of parental females and hybrid offspring [18, 19], it cannot explain the decrease of the progeny number with an increasing proportion of parental males.

In conclusion, strong asymmetry exists not only in the frequency of mating but also in the number of hybrid progeny. As a consequence, the results are nearly the same when one compares those obtained in the normal cross at higher densities and in the reciprocal cross at lower densities. Asymmetry is complex at different levels in the frequency of interspecific mating [11, 20], in the extent of overlap in sexual signals [21, 22], and in the effects of sexual selection [22]. Moreover, there is asymmetry in the numbers of hybrids produced per female which necessarily involves some kind of postzygotic factor.

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Ecdysteroid conjugates in the ovaries of the silkworm, *Bombyx mori*: 3-phosphates of 2,22-dideoxy-20-hydroxyecdysone and of bombycosterol

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Summary. Two novel ecdysteroid conjugates, 2,22-dideoxy-20-hydroxyecdysone 3-phosphate (**1**) and bombycosterol 3-phosphate (**2**), as well as four known ecdysteroid 22-phosphate esters, have been isolated and characterized from the ovaries of the silkworm, *Bombyx mori*.
Key words. Ecdysteroid conjugate; *Bombyx mori*; silkworm; 2,22-dideoxy-20-hydroxyecdysone 3-phosphate; bombycosterol 3-phosphate.

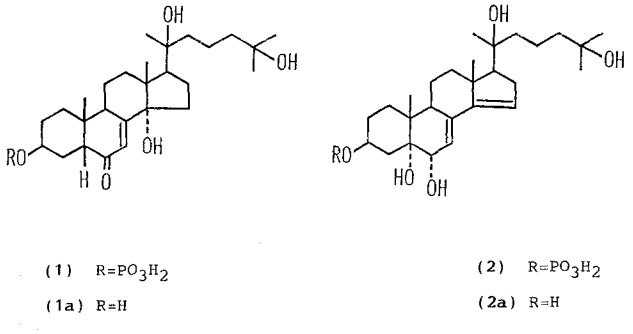

Figure 1. The structures of 2,22-dideoxy-20-hydroxyecdysone 3-phosphate (**1**), 2,22-dideoxy-20-hydroxyecdysone (**1a**), bombycosterol 3-phosphate (**2**), and bombycosterol (**2a**).

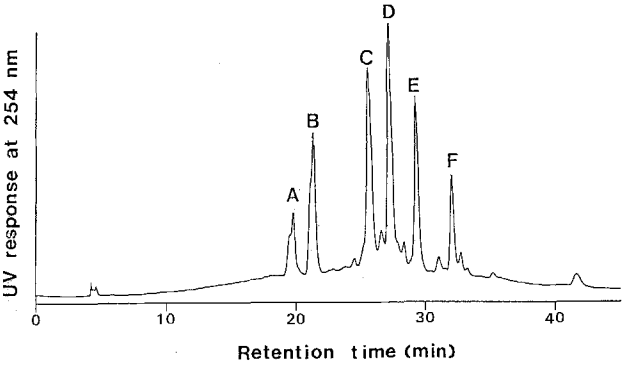

Figure 2. HPLC elution profile of a mixture of ecdysteroid conjugates. The peaks A–F correspond to 20-hydroxyecdysone 22-phosphate, ecdysone 22-phosphate, 2-deoxy-20-hydroxyecdysone 22-phosphate, 2-deoxyecdysone 22-phosphate, 2,22-dideoxy-20-hydroxyecdysone 3-phosphate (**1**), and bombycosterol 3-phosphate (**2**) in the order. Column: Whatman, ODS-3, 0.9 cm i.d. × 25 cm; flow rate, 3 ml/min. Solvent: a gradient of methanol from 5% to 70% in 20 mM sodium phosphate buffer, pH 5.56.

The occurrence of both free and conjugated ecdysteroids in the mature ovaries and eggs of insects is well documented[2]. We previously characterized six free ecdysteroids[3–6], 20-hydroxyecdysone, ecdysone, 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone, 2,22-dideoxy-20-hydroxyecdystone (**1a**), and bombycosterol [(20S)-3β, 5α,6α,20,25-pentahydroxycholesta-7,14-diene] (**2a**) in the ovaries of the silkworm *Bombyx mori*. The last two compounds have so far been found only in the ovaries of this species. In the mature *B. mori* ovaries, conjugates account for a major portion of the total ecdysteroids present[7]. We have now isolated and characterized six ecdysteroid conjugates. Among them, two conjugates have novel structures; viz., 2,22-dideoxy-20-hydroxyecdysone 3-phosphate (**1**) and bombycosterol 3-phosphate (**2**) (fig. 1). The other conjugates are identical to those which have already been isolated from the eggs of the desert locust[8]; viz., 22-phosphates of 20-hydroxyecdysone, ecdysone, 2-deoxy-20-hydroxyecdysone, and 2-deoxyecdysone.

The isolation procedure for the ecdysteroid conjugates from the ovaries was essentially as follows. Ovaries (6000 pairs, about 4 kg wet wt) were dissected from pupae of 1 or 2 days before eclosion, crushed in liquid nitrogen and extracted with aqueous ethanol. The concentrated extract was partitioned between 70% methanol and petroleum ether, and the concentrated methanol layer was passed through a Sephadex G-15 column with ethanol-water 1:9 as an eluting solvent until the yellow color was discharged. The concentrated eluate was then chromatographed on a silicic acid column. Following the elution with benzene-methanol 9:1, which gave free ecdysteroids, elution with methanol afforded an ecdysteroid conjugate fraction. The concentrated methanol fraction was passed through a Sephadex LH-20 column with methanol as an eluting solvent to furnish a mixture of ecdysteroid conjugates at the void volume. Final separation into components was accomplished by high-performance liquid chromatography (HPLC) using a reversed-phase column. The elution pattern is shown in figure 2 (the peaks E and F refer to compounds **1** and **2**, respectively). The amounts of ecdysteroid conjugates obtained were ca 0.7 mg (compound **1**) and 0.6 mg (compound **2**). The isolated compounds **1** and **2** exhibited λ_{max} at 243 nm (ethanol) in their UV spectra.

Upon incubation with intestinal juice from the snail, *Helix pomatia* (suc d'helix pomatia) (Department Reactifs, Pharmindustrie, Clichy, France), compounds **1** and **2** afforded 2,22-dideoxy-20-hydroxyecdysone (**1a**) and bombycosterol (**2a**), respectively, as the sole ecdysteroid. In the negative ion fast atom bombardment mass spectrum (FAB-MS) (fig. 3), each compound exhibited an ion at m/z 527 $[M-H]^-$ which corresponds to a monophosphate ester of 2,22-dideoxy-20-hydroxyecdysone and bombycosterol. Also strong ion peaks characteristic of phosphate ester were observed at m/z 79 (PO_3^-) and 97 ($H_2PO_4^-$).

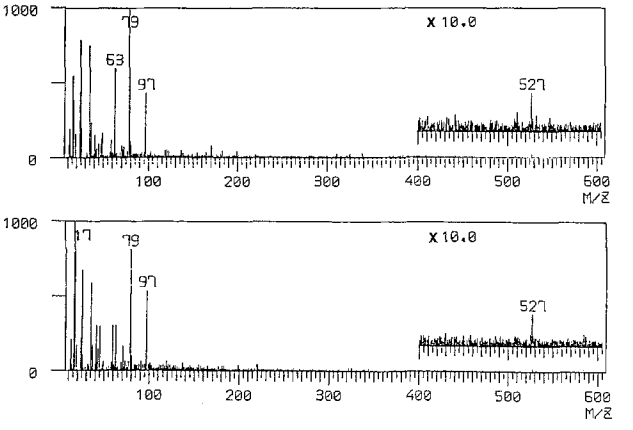

Figure 3. The negative ion FAB-MS of **1** (top) and **2** (bottom).

^{1}H-NMR data (CD_3OD, 200 MHz) of 2,22-dideoxy-20-hydroxyecdysone 3-phosphate (**1**), 2,22-dideoxy-20-hydroxy-ecdysone (**1a**), bombycosterol 3-phosphate (**2**), and bombycosterol (**2a**)[a]

Assignment	(1)	(1a)	(2)	(2a)
3-H	4.51 (m)	4.00 (m)	4.40 (m)	3.89 (m)
6-H	–	–	3.87 (m)	3.89 (m)
7-H	5.80 (d, J=2 Hz)	5.80 (d, J=2 Hz)	5.52 (m)	5.52 (m)
15-H	–	–	5.63 (m)	5.64 (m)
18-H_3	0.85 (s)	0.85 (s)	0.96[b] (s)	0.96[c] (s)
19-H_3	0.94 (s)	0.95 (s)	1.01[b] (s)	1.01[c] (s)
21-H_3	1.27 (s)	1.27 (s)	1.27 (s)	1.28 (s)
26,27-H_6	1.18 (s)	1.19 (s)	1.17 (s)	1.18 (s)

[a] Chemical shifts are given in ppm downfield from TMS.
[b,c] Interchangeable.

The location of the phosphate ester was unequivocally determined by the ^{1}H-NMR data listed in the table. The chemical shifts of all proton signals in the spectra of compounds **1** and **2** are essentially identical to those of the respective free ecdysteroids **1a** and **2a**, except that an oxymethine proton signal ascribable to 3α-hydrogen was shifted downfield by 0.51 ppm in both cases. In the spectrum of **2**, oxymethine signals at 4.40 ppm ($W_{1/2}$ = 28 Hz) and 3.89 ppm ($W_{1/2}$ = 7 Hz) were assigned as 3α-H and 6β-H, respectively, on the

basis of the shape of the signals, since 3α-H is reported to have a larger $W_{1/2}$ value than 6β-H in compound **2a**[9]. Thus, it is apparent that the phosphate group is attached to the C-3 hydroxy group in both cases. Based on the data described above[10], the structures of **1** and **2** were established to be 2,22-dideoxy-20-hydroxyecdysone 3-phosphate (**1**) and bombycosterol 3-phosphate (**2**), respectively. Additional evidence for the structures was obtained by ^{31}P-NMR in which a signal either at 0.92 ppm (compound **1**) or 1.50 ppm (compound **2**), relative to phosphoric acid, was detected. Further, these signals were sharpened to some extent by the selective proton (3-H) decoupling experiments.

The four other ecdysteroid conjugates eluted in the order as shown in figure 2 were identified as 22-phosphates of 20-hydroxyecdysone (peak A), ecdysone (peak B), 2-deoxy-20-hydroxyecdysone (peak C), and 2-deoxyecdysone (peak D) in the same manner as described above. It should be noted that in the ovaries of *B. mori* the ecdysteroids which have a 22-hydroxyl group are substituted at this position to form conjugates, whereas those lacking a 22-hydroxyl group are substituted at the 3-position.

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10 Details of the procedures of their isolation and characterization of these conjugates will be published elsewhere.

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Airborne pollen content in the atmosphere of central Italy (1982–1986)

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Summary. This report describes qualitatively and quantitatively the content of pollen in the atmosphere of central Italy during the five years 1982–1986. Total production in this period showed fluctuations depending on the flowering seasons of the anemophilous taxa. The season of maximum pollen concentration was from April to July, with a prevalence of arboreal pollen in the first months, and of pollen from herbaceous plants in the last months of the year. During the five years of research more than 81 different types of pollen grains were recorded and identified. In both the cities investigated Curpessaceae/Taxaceae, Fagaceae, Oleaceae, Gramineae and Urticaceae were responsible for the greatest amounts of pollen.
Key words. Aerobiology; pollen census; central Italy.

Studies of the pollen content in the atmosphere of different areas have been carried out by researchers in Europe[1–9] and in Italy[10,11]. Surveys of atmospheric pollen grains in two cities of central Italy, Ascoli Piceno and Perugia, have been carried out since 1981 and since 1982, respectively[12–20]. Since the seasonal patterns for all taxa are characteristic, we describe them in terms of annual mean pollen concentrations. Only those taxa with relatively high concentrations were included. In this paper we report the pattern of pollen-production by 12 selected taxa.

Materials and methods. The pollen was collected by 7-day recording volumetric traps[21,22]. The pollen concentration in the atmosphere is expressed in terms of the number of pollen grains per cubic meter of air (p/m^3). The methods used are described in a previous paper[12].

The meteorological data in Ascoli Piceno was recorded by the SIAP Bologna S 2000 meteorological station. In Perugia it was kindly supplied by the Institute of Ecology of the Faculty of Agricultural Science, Perugia University. The meteorological data were plotted as a climatic diagram. Daily pollen concentrations were also noted and were reported on a 5-day mean basis.

Identification of the pollen grains was based on a comparison with reference slides of the Palynotheca of the Plant Biology Department, Perugia University, and photographs which appear in Hyde and Adams[23], Faegri and Iversen[24] and Erdtman[25,26]. The nomenclature of the families, genera and species may be found in Tutin et al.[27].

Pollens of Chenopodiaceae and Amaranthaceae, in addition to those of Cupressaceae and Taxaceae, and Typhaceae and Sparganiaceae, are referred to as Cheno-Amaranthaceae, Cupressaceae/Taxaceae and Typhaceae/Sparganiaceae respectively (table), since they all produce pollen during the same period and are difficult to distinguish, owing to their similar morphology, to the limitations of the methods available, and to the fact that the material is fresh and fixed on the slide[17].

Study area

Perugia (43° 63′ N; 12° 23′ E; 493 m above sea level) lies on a hill and overlooks the middle valley of the river Tevere between Lake Trasimeno and the Tyrrhenian versant of the Marches-Umbrian Apennines. The relative humidity always reaches high values with an average of 70% and the average