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Comparison of the Pollen Content on the Body and in the Gut of Hoverflies (Diptera, Syrphidae)

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Abstract—We analyzed the pollen content in the gut and on the body of hoverflies (Diptera: Syrphidae), mostly from three genera (*Eristalis*, *Syrphus* s. l., and *Sphaerophoria*) that visited flowers of two jointly flowering species of Apiaceae with indistinguishable pollen (*Anthriscus sylvestris* and *Aegopodium podagraria*) and of *Bunias orientalis* (Brassicaceae). Pollen compositions depended on the plant from which the flies were collected rather than on the insect genus. At the same time, individual preferences of the studied flies to food plant species were confirmed. The pollen compositions in the gut and on the body were weakly correlated. Two possible reasons for such a weak correlation are discussed: individual differences in the fly feeding habits and different time of pollen preservation in the gut and on the body.

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Pollination by animals is a well-known example of mutualism among free-living species (Thompson, 2006). These interactions lead to cooperation of plants and their pollinators. At the same time, they also constitute a conflict between the participants, which may result in shift towards antagonistic relations, namely the appearance of deceptive flowers and nectar robbers. It has been shown that the interrelations between plants and pollinators are mostly generalized (Waser et al., 1996): most plants are visited by many pollinator species which, in their turn, may visit many plant species. This absence of specialization is considered to be the inevitable property of any kind of mutualism among free-living species (Thompson, 2006). Due to lack of specialization combined with the inherent selfish nature of relations between plants and pollinators, the participants are not always mutually dependent.

Thus, relations between plants and pollinators are determined by the tasks to be solved by two agents: animals need food while plants need to be pollinated.

One of the most common approaches to the study of the role of insects in pollination is the study of the composition of the pollen transferred by insects on their bodies (see, e.g., Zych, 2007; Lysenkov, 2014). Even insects which do not consume pollen, for instance, obligate nectarivores, may accumulate pollen on their bodies and take part in pollination.

Feeding on pollen (palynophagy) is studied by analysis of the gut content (Dlussky and Lavrova, 2001), and in bees, also by analysis of the pollen pellets (Delaplane et al., 2013).

Anthophily of insects is traditionally studied in bees. Recently, however, the important role of dipterans in pollination has been recognized by researchers (Ssymank et al., 2008; Inouye et al., 2015), although earlier dipterans were regarded as ineffective pollinators (Faegri and van der Pijl, 1982). Hoverflies (Syrphidae) are considered the most important anthophiles among dipterans (Grinfeld, 1978).

The feeding of hoverflies on pollen was first noted by Müller (1873). Pollen diets of hoverflies were studied both by dissection of their intestines (Holloway, 1976; Olesen and Warncke, 1989; Dlussky and Lavrova, 2001) and by examination of their excrements (Holloway, 1976; Golding and Edmunds, 2003). The latter is possible since pollen walls pass intact through the fly digestive system.

At the same time, concurrent investigation of pollen on the body and in the gut of hoverflies was mentioned only by Olesen and Warncke (1989); however, in the cited paper the pollen content was not compared but only the correlation of amounts (not proportions) of the pollen of the same plant species on the body and in the gut of the insect was assessed.

Table 1. The number of hoverflies (Diptera, Syrphidae) of different genera captured on three plant species

Genus of flies	Ashweed (*Aegopodium podagraria*)	Hill mustard (*Bunias orientalis*)	Cow parsley (*Anthriscus sylvestris*)	Total
Eristalis	34	38	22	94
Syrphus s. l.	24	21	12	57
Miyathropa	0	0	1	1
Chrysotoxum	1	1	0	2
Helophilus	0	1	0	1
Leucozona	4	1	2	7
Pipiza	1	0	0	1
Volucella	1	0	0	1
Xylota	0	0	1	1
Sphaerophoria	6	2	12	20
Total	71	64	50	185

The goal of our work was to compare the pollen composition on the body and in the gut of the hoverflies visiting a plot with several species of simultaneously flowering entomophilous plants.

MATERIALS AND METHODS

The study was carried out at Zvenigorod biological research station of Moscow State University (Odintsovsky District, Moscow Province), in a meadow in the floodland of the river Moskva. Hoverflies (Diptera, Syrphidae) were collected during the last week of June in 2012–2014. Three species of entomophilous plants flower most abundantly in the studied meadow at the beginning of summer: the cow parsley *Anthriscus sylvestris* (L.) Hoffm. and the ashweed *Aegopodium podagraria* L. of the umbellifer family (Apiaceae), and also the hill mustard *Bunias orientalis* L. of the crucifer family (Brassicaceae). We recorded on which particular plants the hoverflies were captured.

Anthriscus sylvestris and *Aegopodium podagraria* have similar white inflorescences while *Bunias orientalis* has yellow inflorescences. The composition of the pollinators of the two umbellifer plants is rather similar, and most of the insects visiting them reveal no preference for one of these species (Dlussky, 1998). Besides, the pollen of the cow parsley and ashweed, as well as of most other umbellifers, cannot be differentiated under the light microscope, so that during spore and pollen analysis it is identified only to family (Moore et al., 1991); it is, however, clearly distinguishable from the hill mustard pollen. Therefore, in our analysis we distinguished three types of pollen: “umbellifers,” “hill mustard,” and “other.”

Each captured fly was placed in a separate Eppendorf tube filled with 70% ethanol. In the laboratory, the tube was shaken for several minutes in order to wash pollen off the insect’s body, after which the fly was removed for dissection. Three 50-µl drops of pollen suspension were taken from the tube, each drop was placed under a separate coverslip, and three rows (near the edges and middle of the coverslip) were examined under ×100 magnification; the pollen grains of the three types were counted. If the amount of pollen was small, the whole area under the coverslip was used for counting. The fly retrieved from the tube was dissected in 100 µl of 70% ethanol, the drop containing its gut content was placed under the coverslip, and pollen grains were counted in three rows under ×100 magnification.

For each specimen of Syrphidae, the proportions of umbellifer pollen and “alien” pollen on its body were calculated. For flies collected on the cow parsley and ashweed, the non-umbellifer pollen was considered to be “alien.” For flies collected on the hill mustard, the “alien” category comprised all the pollen except that of the hill mustard.

To achieve a sufficient sample size, the flies were identified only to genus. Most of the captured hoverflies belonged to the genera *Sphaerophoria* Le Peletier, Serville, 1828, *Eristalis* Latreille, 1804, and *Syrphus* s. l. (Fabricius, 1775).

The numbers of specimens of different hoverfly genera captured on different plant species are given in Table 1.

Table 2. Spearman's correlation coefficients for pollen composition on the body and in the gut of hoverflies

Proportion of pollen	*Eristalis*	*Syrphus* s. l.	*Sphaerophoria*	All the flies captured
Umbellifer	0.69*	0.48*	0.11	0.54*
"Alien"	0.66*	0.57*	0.29	0.59*

* Correlation is significant at $p < 0.001$.

Almost all the distributions were essentially non-normal and could not be normalized even by arcsine transformation recommended for analysis of fractions (van Emden, 2008). Therefore, statistical analysis was performed by non-parametric methods. Comparison of proportions of umbellifer and "alien" pollen between plant species and fly genera was made by the Kruskal-Wallis test with subsequent non-parametric multiple comparisons; the relation between parameters of the pollen composition on the body and in the gut was assessed by Spearman's rank correlation coefficient. The distributions were tested for unimodality using Hartigan's dip test (Hartigan and Hartigan, 1985) implemented in the *diptest* package in R 3.2.2; significance was calculated by the Monte Carlo method with 2000 simulations. The remaining statistical analyses were carried out in Statistica 8.

RESULTS

The proportions of umbellifer and "alien" pollen in the guts of flies of the three best-represented genera and also those of "alien" pollen in flies of the genera *Eristalis* and *Syrphus* s. l. were distributed with a statistically significant bimodality (Figs. 1, 2): flies contained either very much or very little pollen of the corresponding type, while intermediate values were almost never observed.

The flies captured on the hill mustard carried significantly less umbellifer pollen and significantly more "alien" pollen than the flies captured on the cow parsley and ashweed, both on the body and in the gut (Fig. 3). This was also evident during separate analysis of the fly genera, although in *Sphaerophoria* this tendency was not significant (Kruskal-Wallis criterion: $p > 0.1$), possibly due to insufficient sample size. At the same time, all the three studied fly genera did not differ significantly in the proportions of umbellifer and "alien" pollen, both on the body and in the gut (Kruskal-Wallis criterion: $p > 0.3$ in all the cases).

In flies of the genera *Eristalis* and *Syrphus* s. l. the proportions of umbellifer and "alien" pollen on the body and in the gut were significantly correlated ($p < 0.001$), though the correlation was not very great (Spearman's coefficient of rank correlation was 0.48–0.69). The correlation in *Sphaerophoria* was non-significant (Table 2), which can hardly be explained by the small sample size since the coefficient was considerably lower than that in the other two genera. The correlation was also preserved when data for all the flies were analyzed together.

For more profound analysis of correlation between the pollen compositions on the body and in the gut, we built a scatter plot of umbellifer pollen proportions on the body and in the gut for flies of the genus *Eristalis*, whose sample was the largest (Fig. 4). The flies mainly concentrated in three corners of the plot since the specimens carrying little umbellifer pollen on the body and a lot of it in the gut were very rare.

DISCUSSION

Bimodality in the pollen distribution testifies to the existence of individual differences in the flies' diets. The proportions of umbellifer pollen show such bimodality only in the gut content, and those of "alien" pollen, also on the fly bodies. Thus, individuals may be subdivided into "chance" visitors (which mainly feed on some plants other than that on which they were captured) and "constant" visitors (which mainly feed on the plant in question). The existence of individual preferences of pollinators has been known for a long time and was referred to as "flower constancy" (Waser, 1986). However, this phenomenon has been mainly studied in bees (Hymenoptera; Apoidea). Bees differ from most pollinators in more developed cognitive abilities, which are believed to facilitate formation of such preferences. There are much fewer indications of flower constancy in Syrphidae: the Scholar Google database as of December 18, 2015 returned 2010 matches to the query *"flower constancy" bee* and only 336 matches to the query *"flower constancy" Syrphidae* (we used the trivial name for bees and the Latin name for hoverflies since it is in such forms that they most often occur in works on pollination). Data on the flower constancy of hoverflies were mainly obtained by studies of their behavior (Goulson and Wright,

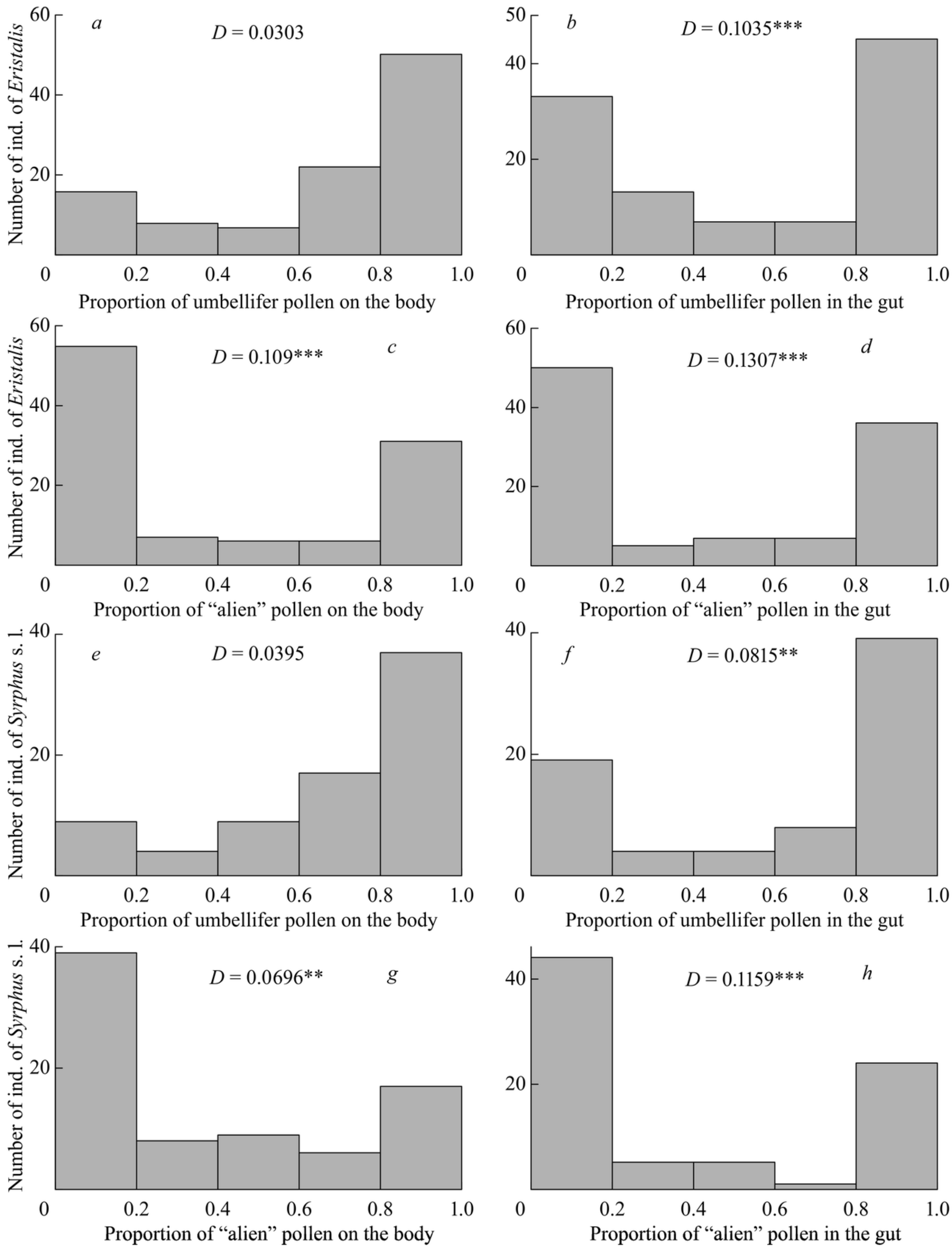

Fig. 1. Distribution of the proportions of umbellifer and "alien" pollen in the gut and on the body of hoverflies: (*a*–*d*) the genus *Eristalis*; (*e*–*h*) the genus *Syrphus* s. l.; (*a*, *e*) proportion of umbellifer pollen on the body; (*b*, *f*) that of umbellifer pollen in the gut; (*c*, *g*) that of "alien" pollen on the body; (*d*, *h*) that of "alien" pollen in the gut. *D* is Hartigan's dip test statistic for unimodality. Significance of deviation from unimodality (calculated by the Monte Carlo method with 2000 simulations): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

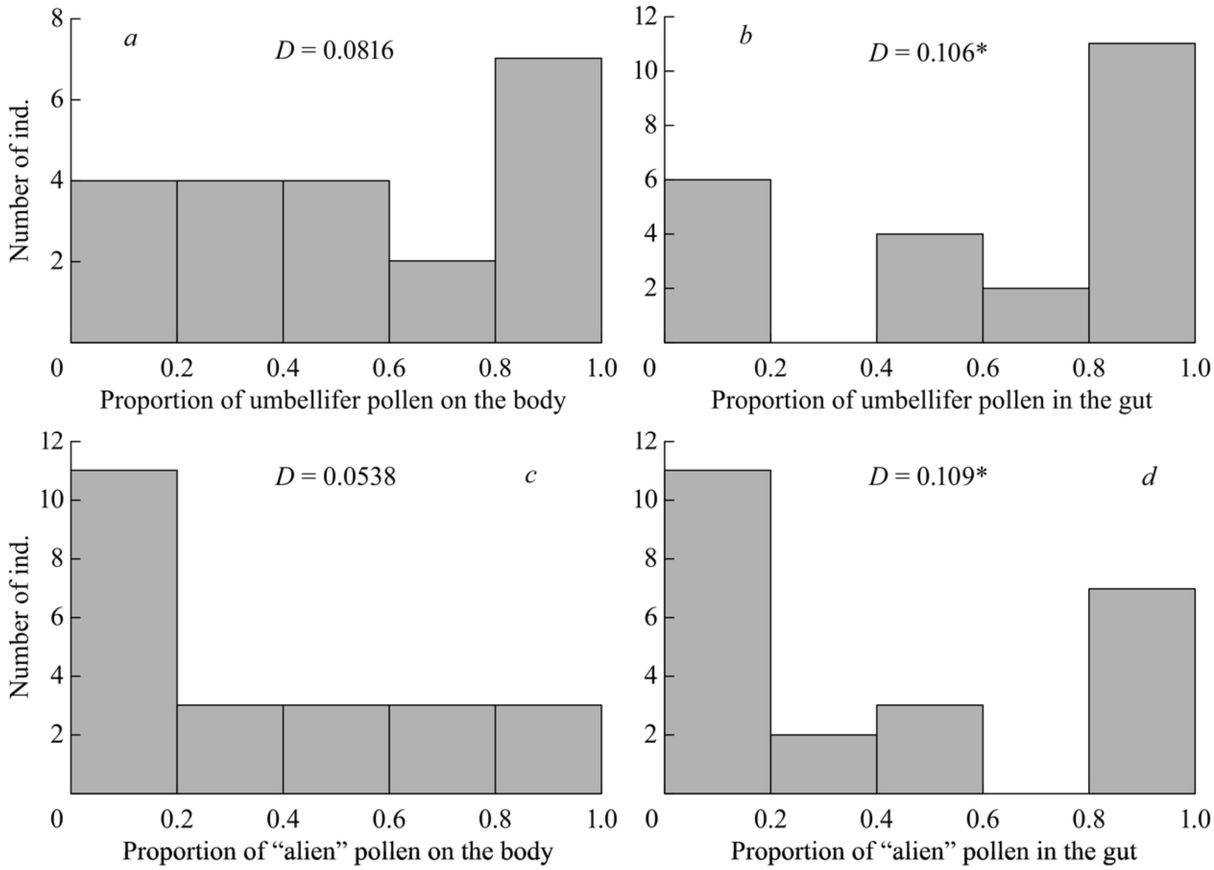

Fig. 2. Distribution of the proportions of umbellifer and "alien" pollen in the gut and on the body of hoverflies of the genus *Sphaerophoria*: (*a*) umbellifer pollen on the body; (*b*) umbellifer pollen in the gut; (*c*) "alien" pollen on the body; (*d*) "alien" pollen in the gut; * deviation from unimodality (calculated by the Monte Carlo method with 2000 simulations) is significant at $p < 0.05$.

1998); besides, Dlussky and Lavrova (2001) suggested the possibility of flower constancy based on analysis of the gut contents. Our work may serve as evidence for flower constancy at least in some hoverflies; however, our data on *Sphaerophoria*, although obtained from a small sample, show a different tendency, namely a great number of specimens with an intermediate pollen composition both on the body and in the gut, suggesting the random choice of plants.

The existence of individual preferences may be also indicated by the fact that the amount of umbellifer and "alien" pollen depends not on the fly genus but on the plant species on which the insect was captured.

We had no possibility to distinguish between the pollen of the two umbellifer species and therefore did not establish whether flies preferred one species to the other. However, comparison of the insects collected on the cow parsley and on the ashweed revealed no difference in the proportion of umbellifer pollen both on the body and in the gut, which confirms the correctness of pooling the corresponding data. Still, it is possible that individual preferences for cow parsley or ashweed do exist in flies.

One of the most interesting results of our research is a weak but still existing correlation between the compositions of the pollen consumed by hoverflies and carried on their bodies. These correlations were not very strong even in *Eristalis* and *Syrphus* s. l. in which the coefficients were statistically significant. As can be seen in the scatter plot, a fairly considerable number of flies showed much inconsistency between the pollen composition on the body and that in the gut. In particular, among flies of the genus *Eristalis* for which the greatest sample was collected (Fig. 4), there were few specimens with a great amount of umbellifer pollen in the gut and a small amount on the body. It is probable that even those flies which do not consume the cow parsley and/or ashweed pollen still visit these plants (for instance, to feed on nectar); but those which consume pollen are sure to carry it.

Differences in the pollen composition on the body and in the gut may result both from different modes of

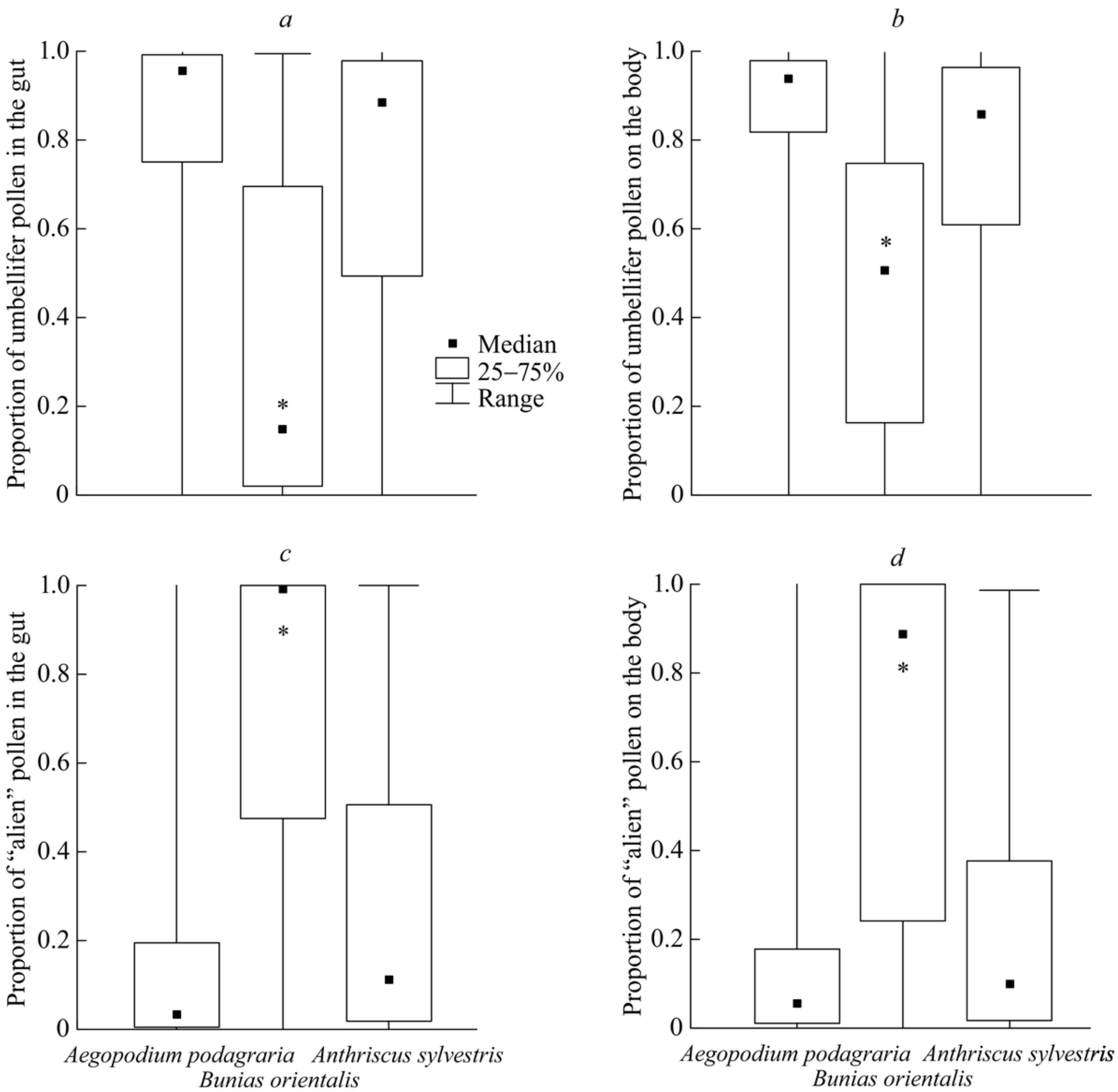

Fig. 3. Composition of pollen on the body and in the gut of hoverflies captured on three plant species: (*a*) proportion of umbellifer pollen in the gut; (*b*) the same, on the body; (*c*) proportion of "alien" pollen in the gut; (*d*) the same, on the body. Significantly different median values ($p < 0.05$, Kruskal-Wallis test with subsequent non-parametric multiple comparisons) are marked with asterisks.

feeding (consumption of nectar or pollen of the given plant) and from different time of preservation of pollen on the body and in the gut. The latter requires additional study; however, pollen seems to remain longer and be renewed slower in the gut than on the body. In particular, according to Schneider (1958, cited after Holloway, 1976), the excrements of overwintered females of the hoverfly *Lasiopticus pyrastri* contained the pollen of not only spring but also some autumn plant species. Thus, the pollen content in the gut may change slower than that on the body and may reflect a longer history of the visited plants.

Differences in the composition of the consumed and carried pollen lead to "asymmetrical" relations between plants and insects. The insect may effectively pollinate plants which are not an important part of its pollen diet and, vice versa, the plant may be ineffectively pollinated by an insect mainly feeding on it. Although such differences occur at the level of individual organisms, they may be important for the evolution of the reproduction system in plants (Hopkins and Rausher, 2012). Asymmetry of mutualism at the species level (with one partner strongly depending on the other but not vice versa) is at present considered an immanent property of such interrelations. Such asymmetry was for the first time demonstrated for plants and their pollinators, using only data on the number of visits (Vázquez and Aizen, 2004).

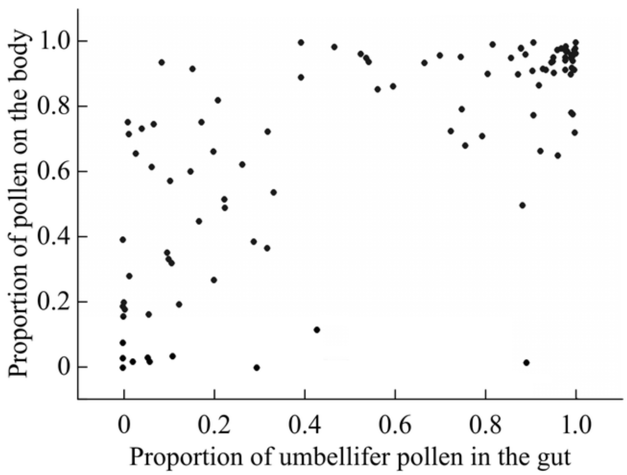

Fig. 4. The proportions of umbellifer pollen on the body and in the gut of hoverflies of the genus *Eristalis*.

Further studies of the pollen composition on the body and in the gut of palynivores would help estimate the degree of interdependence between plants and pollinators and specify the conditions favoring specialization or generalization of their relations.

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