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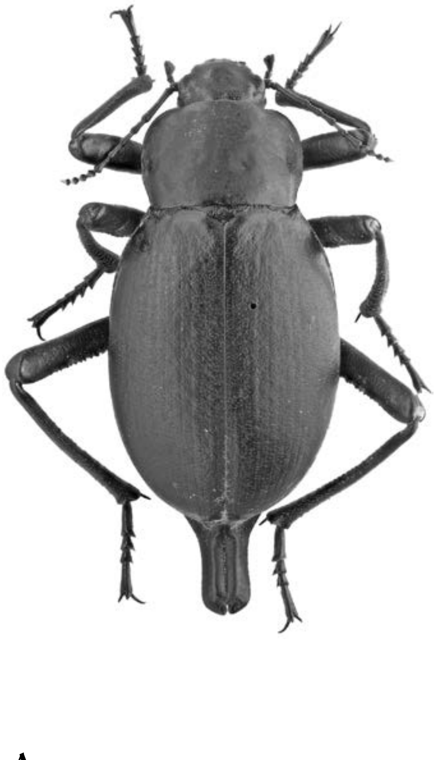

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Duration of visits of several insect species to Geranium palustre flowers

Длительность посещения некоторыми видами насекомых цветков болотной герани Geranium palustre

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Key words: pollination, visit duration, Diptera, *Bombus*, *Pieris napi*, Geranium palustre, insect behaviour.
Ключевые слова: опыление, длительность посещений, Diptera, *Bombus*, *Pieris napi*, Geranium palustre, поведение насекомых.

Abstract. Duration of visits of six insect species to Geranium palustre flowers were assessed using video-records and analyzed with survival analysis which allowed data from incomplete recordings. Median durations vary from 2 seconds in *Bombus pascuorum* to 44 seconds in *Phaonia angelicae* with even more drastic differences in maximum durations. Insects which feed on G. palustre pollen (*Ph. angelicae*, *Helophilus pendulus* and *Rhingia campestris*) spend significantly more time on a flower in male phase than on one in female phase. Some speculations about causes of visit duration differences were done.

Резюме. Длительность посещений шестью видами насекомых цветков болотной герани оценили с помощью видеозаписей. Применение анализа выживаемости позволило использовать данные и по неполным записям. Медианы длительностей варьируют от 2 секунд у *Bombus pascuorum* до 44 секунд у *Phaonia angelicae* с еще более значительной разницей в максимальных длительностях. Насекомые, питающиеся на болотной герани пыльцой (*Ph. angelicae*, *Helophilus pendulus* и *Rhingia campestris*), проводят значимо больше времени на цветках в мужской фазе, чем в женской. Выдвинуты предположения о причинах различий в длительности посещений.

Introduction

Most of animal-pollinated flowering plants are more or less generalized, i.e. visited and pollinated by many animal species [Waser et al., 1996]. Even excluding evident non-pollinators such as nectar thieves and robbers [Inouye, 1980], pollinating visitors differ in their effectiveness and relative importance [Zych, 2002; Larsson, 2005; Ne'eman et al., 2010]. Such variation in pollination service from different species is a prerequisite for the evolution of flowers: natural selection tends to modify flowers to increase reproductive success in local conditions with the specific set of important pollinators [Vakhrameeva, Dlussky, 1994; Mitchell et al., 2009; Beans, Roach, 2015]. Thus, study of differences in visitor behavior on flowers is crucial to understand functioning and evolution of generalized plant-pollinator systems.

There are different approaches to assessing relative importance of different pollinators. Direct evaluation of pollen removal and deposition are often too laborious [Zych, 2002] so different indirect methods are used. Often in such studies pollinator species are combined into so called "functional groups" (e.g., beetles, hoverflies etc.). Such grouping is inevitable but possible intra-group differences are neglected under this approach. Video recording is a good tool to explore behavior peculiarities of pollinators [Edwards et al., 2015], especially in generalist plants with usually open flowers which are easy to observe.

Visit durations can be used as an estimation of the pollinator's involvement in an interaction with the plant and can be correlated with pollen removal and deposition. In this short communication data on visit durations and floral behavior of several visitors belonging to different "functional groups" of a generalist plant Geranium palustre as a case study obtained with video-recordings are analyzed providing new information about their roles in pollination of this plant. Because it is often difficult to record whole visits of every insect especially when the researcher should wait until it sits on a flower, survival analysis taking into account such incomplete data were used.

Material and methods

Study species and site. Geranium palustre L. (Geraniaceae) is a herbaceous perennial plant. Its flowers are upward directed, "pleiomorphic" (flat and radially symmetric with few symmetry axes), with five purple petals, sexual organs are erected in center of the flower [Sugorkina, 1995; Kozhuharova, 2002]. Flowering season is long: it begins in end June-beginning July and lasts till end August-mid September [Sugorkina, 1995]. Geranium palustre is protandrous: stigma opens and becomes receptive only after end of anthesis. Its open flat flowers with usually bright corolla are accessible for almost all anthophilous insects. Dlussky et al. [2000] asserted that G. palustre is visited by many insects from different "functional groups" (bees, flies and butterflies), but main pollinators are flies and small bees because larger bees fall from a flower due to bending stalk and butterflies suck nectar sitting far from

Table 1. Number of registered visits by year of observations and by insect identity. Number in parentheses shows number of censored observations (data only on partial visit duration available). Zeroes indicate that data on the given species were not collected during this year.

Таблица 1. Число зарегистрированных посещений цветков болотной герани разными насекомыми в годы наблюдений. Числа в скобках показывают число цензурированных наблюдений (доступны данные только по неполной длительности посещения). Нули обозначают, что в этот год наблюдения данного вида не проводились.

Year / Год	Insect / Насекомое						
	Phaonia angelicae	*Rhyngia campestris*	*Bombus pascuorum* workers рабочие	*Helophilus pendulus*	*Sphaerophoria menthastri*		*Pieris napi*
					males / самцы	females / самки	
2010	42 (0)	0	0	105 (0)	0	0	56 (0)
2011	0	0	0	143 (24)	157 (19)	113 (13)	103 (8)
2013	47 (14)	25 (5)	124 (12)	37 (7)	0	0	90 (12)
Total	89 (14)	25 (5)	124 (12)	285 (31)	157 (19)	113 (13)	249 (20)

anthers and stigma. But their suggestions of pollination effectiveness were done based only on a few observations (1–3 per species).

Studied G. palustre plot was situated in the wet grassland at high-water bed of the Moscow River near MSU Zvenigorod Biological Station (Odintsovo District, Moscow Region, Russia), coordinates 55°42′18.7″N / 36°44′35.2″E. The peaks of flowering in this plot were in the August during all observed years.

Insect visitation observations. Several species of interest were selected among all insect visitors based on their relative abundance allowing collecting sufficient sample and representing different functional groups of anthophilous insects. The choice was based on abundance of the species and easiness to identify it without collecting. These species made substantial part of all insect visits to G. palustre flowers. The species used in this study are following: 1) worker *Bombus pascuorum* Scopoli, 1763 (Hymenortera: Apidae); 2) *Pieris napi* Linnaeus., 1758 (Lepidoptera: Pieridae); 3) *Phaonia angelicae* (Scopoli, 1763) (Diptera: Muscidae); 4) *Rhyngia campestris* Meigen, 1822 (Diptera: Syrphidae); 5) *Helophilus pendulus* (Linnaeus, 1758) (Diptera: Syrphidae); 6) *Sphaerophoria menthastri* Vockeroth, 1963 (Diptera: Syrphidae).

Every year data have been collected in August during peak flowering of G. palustre in the above-mentioned site. Observations took place before noon during peak visitation.

Some preliminary data were got in August 2010 during insect visit observations with a stop-watch. Main data were got in August 2011 and 2013 when insect visits were video recorded with Nikon DX-300. The observer stood near geranium flowers waiting for insect visitors. Having remarked an insect of interest he began video recording. If the moment of visit beginning were remarked, the time from the beginning of visit to the beginning of recording in seconds [e.g. “Landed three seconds ago”] was said on a camera. If visit beginning was unknown no indication was made. Sequential visits were recorded without interruption if possible.

Durations of individual visits were determined from video recordings from the moment of landing till the moment of departure from a flower. Sexual phase of visited flowers was also remarked except they were too far to determine it properly. In *Sph. menthastri* insect sex was also remarked.

Sample sizes are shown in the Table 1.

Statistical analysis. Because substantial part of registered visits was recorded not from the beginning, survival analysis was decided to be used for comparison of visit durations. This method takes into account so-called “censored observations” when the full duration of a studied process is unknown but it is known that it took no less than some particular time. It is based on median rather than mean because the latter parameter could not be estimated from censored data.

There is no widely accepted test for two-sample comparison of survival data, so as a proper multiple comparison test. Data on different applicability of three widely accepted tests (log-rank, Gehan’s and Cox-Mantel) are also controversial; in general Gehan’s test seems to be less powerful as two others [Lee et al., 1975]. All three tests were used in this work and in all cases except one (see Results) they gave consistent results.

For every insect species durations of visit on male and female flowers were compared.

Inter-species comparisons were made with Bonferroni-corrected two-sample tests to avoid multiple comparison error.

For graphical illustration of differences in visit duration we used “cumulative survival plot” reporting proportion of insect individuals spent on a flower no less than specific time.

All statistical analyses were made with STATISTICA 8.0 software.

Results

Visit durations have right-skewed distributions in each species: most individuals tend to spend little time on a flower, whereas some of them spend much longer.

Sphaerophoria menthastri females and males do not differ in visit durations ($p > 0.6$ in all three two-sample tests) so data on them were combined. Overall inter-species differences in visit duration are significant (Chi-square = 371, df = 5, $p < 0.001$). Visit durations show great inter-species variability, an order of magnitude (Fig. 1): median time varies from 2 seconds in *B. pascuorum* to 44 seconds in *Rh. campestris* (Table 1). Bumble bees *B. pascuorum* spend the least time on a flower, the longest visit lasted only 7 seconds. All three hoverfly species have different visit durations: from median 6 seconds in *H. pendulus* (so as butterfly *P. napi*) to 44 seconds in *Rh. campestris*. Muscid fly *Ph. angelicae* and above-mentioned *Rh. campestris* have the longest visit durations, with statistically insignificant differences between them.

Insects of some species spend more time on flowers in male phase than on flowers in female one: *Ph. angelicae* ($p < 0.009$ in all three tests), *H. pendulus* ($p < 0.005$ in all three tests) and, possibly, *Rh. campestris*. In the last case the least powerful Gehan's test does not show significant differences ($p = 0.14$) whereas two other do ($p = 0.04$ in log-rank test and $p = 0.02$ in Cox-Mantel test). It can be seen from Fig. 2 that differences comes from several visits which spent near 10 min on male flowers whereas durations of most other visits didn't exceed 2 min. But differences in visit durations between "long-sitting" hoverflies (*Ph. angelicae* and *Rh. campestris*) and "medium-sitting" ones (*Sph. menthastri*) remain significant if only visits to female-phase flowers are taken into account ($p < 0.05$ in all three two-sample tests).

Such huge differences in visit durations make us to suggest that *Rh. campestris* and *Ph. angelicae* can spend most of this time without special activity on a flower. Exceptionally long visits of these insects (more than 90 seconds) were re-watched more carefully to assess duration of insect activity. Most of the time flies were active contrary to the suggestion (Table 2).

Discussion

Huge, an order of magnitude, differences in visit durations were detected even in species usually treated as belonging to the same "functional group", e.g. all three hoverflies have significantly different median visit duration. It should be stressed that no one of the studied insect species are unusual visitors of G. palustre. Similar

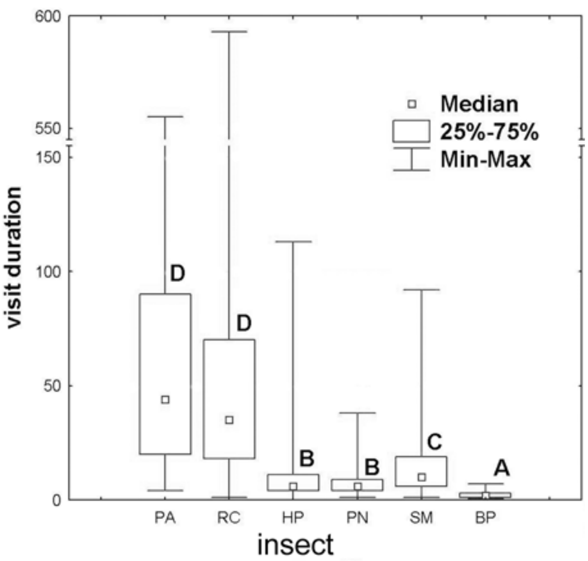

Fig. 1. Insect visit durations to Geranium palustre flowers. Significantly different durations do not share any letter.

Рис. 1. Длительность посещений насекомыми цветков болотной герани. Значимо различающиеся длительности не имеют общих букв.

BP – *Bombus pascuorum* (Hymenortera: Apidae); PN – *Pieris napi* (Lepidoptera: Pieridae); PA – *Phaonia angelicae* (Diptera: Muscidae); RC – *Rhyngia campestris* (Diptera: Syrphidae); HP – *Helophilus pendulus* (Diptera: Syrphidae); SM – *Sphaerophoria menthastri* (Diptera: Syrphidae).

Table 2. Passively spent time by pollinators of G. palustre during extremely long (more than 90 s) flower visits. An insect was treated as passive if it were not moving on a flower and were not feeding.

Таблица 2. Пассивно проведенное время опылителями болотной герани во время экстремально долгих (более 90 с) посещений. Насекомое считалось пассивным, если не двигалось и не питалось на цветке.

Insect species Вид насекомого	Total visit duration, s Общая длительность посещения, с	Passively spent time Пассивно проведенное время	
		Time, s Время, с	Proportion, % Доля, %
Phaonia angelicae	139	8	6
	171	61	36
	212	134	63
	167	0	0
	104	0	0
	150	0	0
	122	0	0
Rhyngia campestris	593	24	4
	569	16	3
	340	93	27
	309	39	13

differences though seemingly not so drastic were detected in visit duration by different hoverflies to Cornus canadensis [Edwards et al., 2015] – unfortunately statistical analysis of significance of these differences was not reported in the cited work.

The shortest visit duration was in bumble bees. The longest of recorded visit didn't exceed median of three other species and only slightly exceeded median (and lower quartile) visit duration of *P. napi* and *H. pendulus*. Such short visits can be caused by the fact that flexible flower stalk of G. palustre throws down large insects which is hypothesized to be a mechanism to limit pollinator range [Dlussky et al., 2000]. But it should be stressed that such disturbance didn't prevent bumble bees from following visits to G. palustre flowers. Its mean number of registered sequential visits = 10.5 (range 3–20), only *Ph. angelicae* and *Rh. campestris* has significantly different (lower) mean number of registered sequential visits (Fisher LSD, Tukey HSD and Sheffe post hoc tests in ANOVA gave consistent results). Thus, during the same time a bumble bee can visit (and does visit) more flowers than other insects.

Extraordinary long visit durations of *Ph. angelicae* and *Rh. campestris* have no clear explanation. These two species belong to different families considered to be different "functional groups" (Muscidae and Syrphidae, respectively). Most part of a visit these flies are active moving on a flower and feeding on nectar or pollen. Two times *Ph. angelicae* in 2013 (no such data from 2010 available) moved to the underside of a corolla and sat there for a long time (95 and 30 seconds). Similar behavior was seen in other species only twice (in *H. pendulus* for 4 seconds and in *P. napi* for 4 seconds; take into account greater sample sizes for these species). Bumble bees several times also sat on underside of a flower but they fed on it during these unusual visits. In 2010 it was seen several times that *Ph. angelicae* drove away larger *H. pendulus* who had sat down on the same flower. *Phaonia angelicae* also tend to visit G. palustre flower which are situated separately from the dense central part of the plant patch [Lysenkov, 2009]. So it can be suggested that some kind of territorial behavior takes part

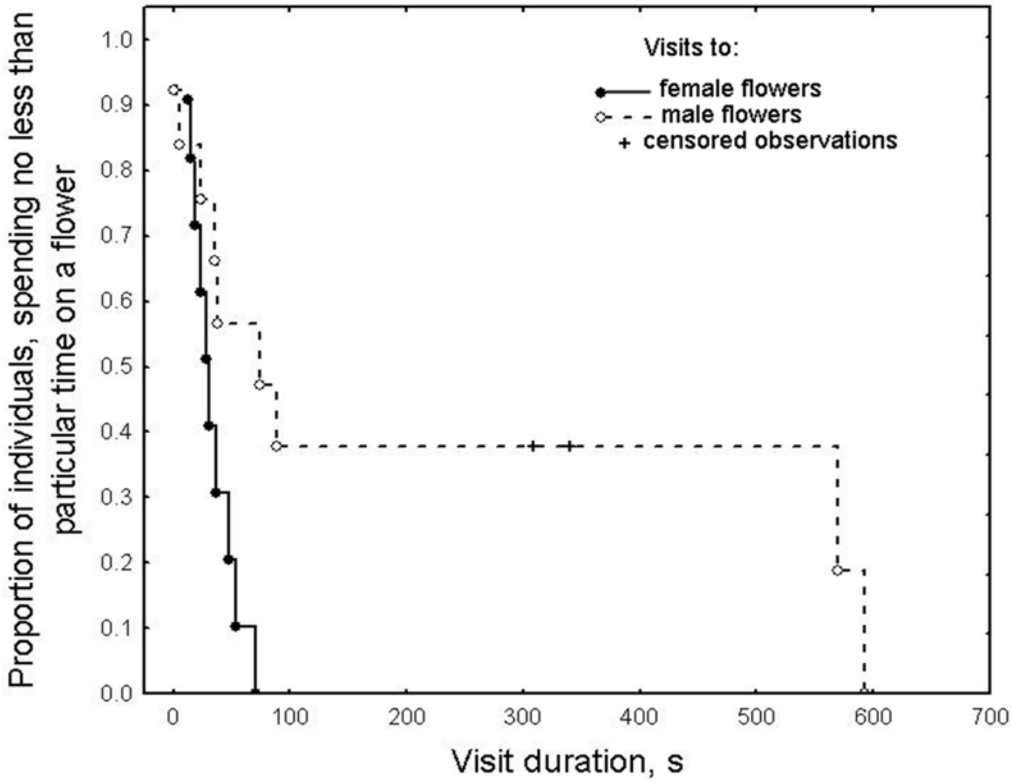

Fig. 2. Cumulative visit durations of *Rhyngia campestris* to Geranium palustre flowers in two sexual phases: male (dashed lines, open circles) and female (solid line, filled circles). Crosses mark censored observations (actual visit duration were nor less than the time).

Рис. 2. Накопленные длительности посещений *Rhyngia campestris* цветков болотной герани в двух половых фазах: мужской (пунктир) и женской (сплошная линия). Кресты отмечают цензурированные наблюдения (истинная длительность посещений была не меньше, чем отмеченное время).

in such long visits of this muscid fly. But no such clues were found in *Rh. campestris* which spent most time on a flower feeding with its long proboscis.

Three pollen-feeding species (all flies except *Sph. menthastri*) spend significantly more time on male-phase flowers than on female-phase ones. Usually flower visitors increase visit duration when encounter more rewards [Neff, Simpsons, 1990; Manetas, Petropoulou, 2000]. In our case this can be resulted either from presence of additional food source (pollen) and/or greater nectar production in male-phase flowers. Lack of such differences in *Sph. menthastri* which is also a palynophagous but feed only on nectar in G. palustre [Dlussky et al., 2000; personal observation] allows us to consider that the cause of difference is the pollen presence. Grinfeld [1978] reported that pollen-feeding beetles spent more time on flowers than nectar-feeding.

Effect of different visit duration on plant fitness is usually reported to be positive due to increasing pollen removal and deposition [Conner et al., 1995; Wolff et al., 2006; but see Rush et al., 1995]. Perhaps long visits can explain why *Ph. angelicae* individuals from G. palustre have the same pollen load as larger fly *H. pendulus* [Lysenkov, 2014a]. On the other side, Dlussky et al. [2000] reported that *H. pendulus* deposit several tens of pollen grains whereas *Rh. campestris* less than ten, but these data were based only on a few observations, no data on visit durations were also shown. It is seen on the video recordings that *Ph. angelicae* and *Rh. campestris* from time to time climb on the stigma, this behavior can possibly bring about greater pollen deposition but further researches are needed. Though bumble bees spend only few seconds on a single flower they can cause substantial pollen flow because evidently touch anthers and stigmas and visit several times more flowers per unit time than other insects. *Pieris napi*, *H. pendulus* and *Sph. menthastri* spend comparable time on a single flower. The former species seems to be nectar thief or very inefficient pollinator because it touches anthers and stigmas very seldom [Dlussky et al., 2000; personal observation] due to small size. *Pieris napi* sit on the petal but often touch anthers and stigmas by head, proboscis and legs during feeding in contrast to assertion from the above cited work that butterflies do not contact sexual organs during flower visit (possibly because it was based on observations on smaller Hesperiidae butterflies) so their role in G. palustre pollen transfer could be substantial taking into account relatively high visitation rate (due to small visit duration) and long inter-visit flights [Lysenkov, 2014b]. Video recordings confirmed data of Dlussky et al. [2000] that *H. pendulus* during visit actively contact anthers and stigmas. Their role in pollination should be greater than other studied relatively large flies because of small visit duration and, hence, high rate of pollen transfer.

Such huge differences in visit duration between even relatively close insect species, usually combined in one "functional group", should be taken into account during researches on relative pollinator importance. E.g., in the paper of Conner et al. [1995] visit duration of Raphanus raphanistrum by "syrphid flies" without further subdivisions showed the greatest variability of all studied pollinators (honey bees, small bees and butterflies). Possibly, part of this variation is explained by inter-species differences.

Visit duration data are also needed to be taken into account during transect visitation rate studies: long sitting insects can be overestimated due to higher probability to be remarked on a flower.

As a result of this study, survival analysis of the visitation video records looks plausible method for the investigation of pollinators' visit durations. These data could be taken into account for estimating pollination effectiveness and relative importance of different species within a "functional group".

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