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DEVELOPMENT AND NESTING BEHAVIOUR OF TRAP-NESTING WASPS (HYMENOPTERA: VESPIDAE: EUMENINAE)

Summary of doctoral dissertation

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The dissertation is available in the library of the Vilnius University.

INTRODUCTION

Relevance of the study. Aculeate Hymenoptera are essential ecosystem components that act as pollinators and predators (Lassalle & Gauld 1993). They provide examples of the most sophisticated reproduction and brood care behaviour among invertebrate animals (Ayasse *et al.* 2001).

Since Hymenoptera Aculeate are known to be bioindicators, which are sensitive to environment change (Tscharntke et al. 1998), management and succession (Gathmann *et al*. 1994), diversity (Gathmann & Tscharntke 1999) and fragmentation (Morato 2001, Steffan-Dewenter *et al*. 2002) of habitats, antropogenous impact and climate change (Pekkarinen & Hulden 1991), these insects may have an importance in environment conservation.

Most species in the family Vespidae belong to the subfamily Eumeninae (Carpenter 1986). These wasps are known to control populations of leafrollers (Klein 2004; Harris 1994; Yamane 1990; Jennings & Houseweart 1984), leaf beetles (Schenk & Bacher 2002, Sears *et al*. 2001) and weevils (Bohart et al.1982).

Eumeninae is a sister group of the subfamilies Stenogastrinae, Polistinae and Vespinae of the family Vespidae (Carpenter 1981; Brothers 1999; Carpenter & Wheeler 1999; Hines *et al*. 2007). It was used as an outgroup in the phylogenetic studies of these social wasps. Thus, study of reproductive behaviour and behavioural ecology of Eumeninae can provide important data for understanding the evolution of sociality in Hymenoptera.

Goal and objectives. The purpose of our study was to establish impact of nesting cavity parameters and prey abundance on the nesting behaviour of solitary xylicolous wasps.

To achieve this goal, we set up the following objectives:

- to survey a list of Hymenopteran species inhabiting trap-nests in Lithuania and estimate how the choice of a nesting cavity is affected by the width of a nesting cavity in different species;
- to estimate the impact of nesting cavity parameters (diameter and length) on the nesting behaviour of trap-nesting wasps;

- to detail the development of *Symmorphus allobrogus*, the most common synanthropic xylicolous wasp in Lithuania;
- to estimate the influence of prey quantity on the nesting behavior of *S. allobrogus*;
- to evaluate the impact of the wasp's individual parameters such as age, body size and insemination status on its nesting behaviour.

Novelty of the study:

- for the first time, the influence of nesting cavity parameters (width and length) on the nesting behavior of 7 to 13 xylicolous wasp species has been estimated: the relation between cell length, progeny size, sex ratio and the nesting cavity diameter has been established, as well as the relation between the number of cells and nest length has been investigated;
- for the first time, the ranges of the head width of all five instars in 5 wasp species have been established;
- for the first time, the development of the most common synanthropic xylicolous wasp in Lithuania, *Symmorphus allobrogus*, has been investigated: the assimilation of prey and weight loss during development from larva to adult in both sexes have been compared;
- for the first time, using *S. allobrogus* as a model species, it has been demonstrated that the amount of prey available in the environment affects the number of cells in the nest of solitary wasp;
- for the first time, the nests of virgin solitary wasp females have been attained under laboratory conditions.

Significance of the results. The results of the study may be important for future ecological and ethological studies of cavity-nesting Hymenoptera as well as for application of trap-nest technique in Hymenoptera research:

- the established preferred nesting cavity parameters may be applied in the species niche studies;

- the established dependence of the number of recorded species on sampling effort in a study site can be applied in comparative studies of species diversity using trap-nests for Hymenoptera;
- the estimated influence of nesting cavity parameters on nest structure and sex ratio of studied species may be used in further studies of their nesting behaviour;
- the established width of head and the parameters of larval development of 5 species may be used for approximate estimation of wasp nest age;
- the established parameters of prey assimilation during the larval development and weight loss during development to adult wasp may be applied for estimation of the amount of consumed prey, using adult weight of a wasp;
- the established relation between amount of available prey and cell number per nest in laboratory as well as effect of wasp's aging on its nest structure may be applied in comparisons of ecosystem parameters using trap-nests for Hymenoptera in the field.

Presentation and approval of the results. The materials of the dissertation were presented at the conference "Biodiversity, Molecular Ecology and Toxicology" (Palanga, Lithuania, 2005). The results have been published in 3 articles (1 published and 2 accepted for publication in 2009); other 2 have been submitted.

Structure of the dissertation. The dissertation consists of the following parts: Introduction, Review of Literature, Material and Methods, Results and Discussion (containing 4 sub-chapters), Defended Conclusions, References (133 positions) and a list of the author's publications promoting the results of the dissertation. The dissertation has 97 pages, containing 21 tables and 41 figures. It is written in Lithuanian, with its summary in English.

Acknowledgements. First and foremost, I am greatly indebted to my supervisor, dr. Eduardas Budrys. This thesis would not have been possible without his substantial contribution in generation of ideas, preparation of publications and identification of insects. His abiding commitment to knowledge has been an inspiring example and I am grateful to him for his supervision and mentorship during all my studentship.

This thesis would not have been possible without dr. Anna Budrienė, who prepared the wasps for the experiments in laboratory. I also extend my heartfelt thanks to

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Last but not least, I would like to give my special thanks to my family and friends, whose love and support enabled me to complete this work.

Review of literature. Herein available knowledge of nesting ecology of xylicolous wasps as well as available surveys of their nesting parameters and influence of mother body size on reproductive success of Aculeata are reviewed. Also a survey of application of trap-nesting Hymenoptera in various studies is presented.

MATERIAL AND METHODS

Material. The field research was carried out in Lithuania during 2002-2007 in 14 sinantropic sites with relatively similar rural or green urban environments (Table 1).

We used internode fragments of reed (*Phagmites australis*) stems as trap-nests. The trap-nests were exposed on old wooden or daubed buildings inhabited by colonies of cavity-nesting solitary Hymenoptera. They were exposed from May to the end of August. A trap-nest exposed during one summer season was considered a sampling unit (a trap-nest-season). In the three main sampling sites (Table 1), the trap-nests were checked few times per season, and either the occupied reed internodes with visible external plugs were replaced by new internodes or the whole trap-nest was replaced by a new one. Thus, the trap-nests were never completely filled. Newly plugged

Table 1. Trap-nest sampling sites, periods and effort.

Site	Coordinates	Years of sampling	Sampling effort (trap-nest-seasons)
Varnupys	55°24'N 25°17'E	1989-2007	137
Papiškiai	55°56'N 24°16'E	2002-2004, 2007	37
Bilšiai	55°08'N 25°16'E	2001-2007	36
Kaunas	54°54'N 23°54'E	1989-1991, 2000	7
Taraldžiai	55°46'N 25°22'E	2003-2004	7
Merkinė	54°10'N 24°10'E	1989-1992, 2000	6
Kiemeliai	54°51'N 25°01'E	2007	4
Paburgė	56°01'N 21°56'E	2007	4
Puvočiai	54°07'N 24°18'E	2007	4
Trečiokiškės	54°50'N 24°59'E	2007	4
Subartonys	54°12'N 24°11'E	2003	3
Pylimėliai	54°43'N 25°21'E	2003	2
Veržuva	54°45'N 25°26'E	2003	2
Antagavė	55°19'N 26°09'E	1989	2
		Total	255

internodes were dissected longitudinally for nest cavity measurements and study of the nest content. The parameters measured directly were the internal diameter and depth of a reed internode with a nest and the depth of each diaphragm separating brood cells. Also we counted and weighed the prey in each cell if it still was not consumed. The sex of wasps was determined at the pupal stage. The diapausing prepupae or the adults of wasps and bees were placed into a refrigerator (4°C) for a period of 2 – 5 months to mimic hibernation. Afterwards, the larvae were preserved at room temperature until they developed to adults. The sex of wasps was determined at the pupal stage.

To assess the relation between the number of species per site and sampling effort we used logistic regression.

Equipment. The estimations were made using a binocular MBS 10 and Nikon SMZ800, at a magnification 32 x. The wasps and their prey were weighed using torsion balances BT (Kiev) (readability 0.3 mg) and electronic balances Kern ABJ (readability 0.1 mg). The data were processed using the database-managing system Microsoft Access 2000. Statistical analysis was performed using the computer programs StatSoft STATISTICA, release 6.0. and SAS, release 9.1.

Effect of nesting cavity parameters on nesting behaviour For testing the hypotheses considering the dependence of cell length on the nesting cavity width, we used Pearson correlation. Female brood cells are considerably larger than male brood cells, therefore, the cells of each sex were analysed as separate datasets.

To estimate the influence of nesting cavity parameters on sex ratio, progeny size, cell number per nest and the area of diaphragms that fall on a unit weight of progeny we used Spearman correlation.

Measurement of the optimal parameters of nesting cavity. We considered the parameter as proper for estimation of the optimal diameter or depth if its dependence on diameter or depth of the nesting cavity had a pattern of binomial regression ($y = ax^2 + bx + c$). Density of cells was calculated by dividing number of cells by the length of nesting cavity. The usage of the nesting cavity was calculated by subtracting the length of nest itself from the depth of the nesting cavity.

Development of *S. allobrogus*. We measured egg length and weighed provision in each cell of newly built nests. Wasp larvae were weighed and head width was measured;

some larvae were weighed and measured repeatedly every second or third day until start of building the cocoon. Prepupae (larvae after defecation) were weighed before and after reactivation; pupae and the newly hatched imagoes were weighed as well.

In order to estimate the dependence of weight increment on the larval weight itself, we considered the average weight of two successive weight measurements as an independent variable and the same average weight divided by the time interval between these two measurements (in terms of days) as a dependant variable.

Study of the effects of prey quantity on the nesting behaviour of *S. alobrogus*. A total of 96 *S. allobrogus* females were used in the experiment. 81 wasps were reared in laboratory from trap-nests that have been exposed in Varnupys, Bilšiai and Papiškiai (Table 1). The other 15 females, in 2008 spring, were captured in the places mentioned above. As long as these females failed to copulate in captivity, we considered them as mated in field.

The experiment was conducted in the laboratory from 2006 to 2008, in transparent plastic 5 litre containers. The containers were kept at ambient temperature with food (honey solution and water), nesting places (reed internodes) and material for building cell partitions (clay) available. The prey larvae were exposed on small branches of the host plant. The content of newly built nests was examined.

In our experiment, we used the third instar larvae of the natural prey of *S. allobrogus*, leaf-beetles *Gonioctena quinquepunctata* and *Linaeidea aenea*. The larvae of the former species were collected in field on the bird cherry (*Padus avium*) and the european rowan (*Sorbus aucuparia*). The larvae of *L. aenea* were reared in the laboratory on the grey alder (*Alnus incana*) from the eggs laid by adult beetles collected in the field.

Females were treated in one of the two ways: they either received a fixed amount of prey - 5, 10 or 20 prey individuals daily during the experiment (permanent regime), or the amount of prey amount was alternated between 5 and 20 individuals per day, after every second nest had been finished (shifting regime). Ten waps under shifting regime stated with 5 preys per day and eleven wasps — started with 20 preys per day.

Virgin females were placed into containers on the same day they had hatched or 1-12 days later. Other females were placed into containers immediately after mating in lab or 1-18 days after that (for more details of the mating procedure, see Budrienė 2004).

In the first experimental year, we kept the females inside of the containers until their death to evaluate nesting dynamics. It appeared that it takes from 1 to 14 days to start nesting. Some wasp females died in a relatively short time without demonstrating any nesting activity. Most of the females demonstrated gradual decrement of nesting activity with their age, as long as they stopped supplying the nest with any larva and eventually died. The period from nesting activity termination to death was 10 - 17 days. In the light of these remarks, in the next two experimental years, a total of 16 females that had did not demonstrate any nesting activities were eliminated from the experiment after 14 days, and all remaining females were eliminated 7 days after the termination of nesting activity.

In order to use parametric statistical methods, the data (cell number per nest) were transformed using Normal approximation to a Poisson distribution (Kruopis 1993).

We used the analysis of covariance (ANCOVA) to assess differences in the amount of cells per nest between groups of prey quantity in a permanent regime. We used trap-nest length, head width and body weight of a female wasp as concomitant variables. The primary analysis demonstrated that the regression lines were not parallel for the nesting cavity diameter in the three prey quantity groups (F=4,14; P=0,02), therefore we didn't use nest cavity diameter as concomitant variable in further analysis. Multiple comparisons were made using Scheffé test. For the nests made in a shifting regime, differences in the amount of cells per nest between groups of prey quantity were tested using a mixed model for repeated data (we used only 1st-8th nests of each wasp). In the case of virgin females we used Student criterion for independent samples.

Neither the cell number amount per nest, nor prey weight in a cell manifested a difference, while using the two different species of prey (Student criterion, Mann-Whitney U test), therefore we pooled data and analyzed it as a single dataset. We did not detect any significant differences in the prey weight per cell or sex ratio in the nest made under permanent and shifting regimes (Mann-Whitney U test), therefore we analyzed the aforementioned data irrespective of the character of the regime. To assess the differences in prey weight per cell, progeny size and portion of females (calculated as portion of female progeny per nest and as female progeny made per one wasp during the lifelong experiment) in different prey quantity groups, we used Kruskal-Wallis, Mann-Whithey U and Wald-Wolfowitz tests.

The total number of cells made by a wasp was calculated only for the females, which were kept under experimental conditions (permanent regime) during all their life.

RESULTS AND DISCUSSION

Solitary Hymenoptera inhabiting trap-nests in Lithuania

Table 2. List of cavity-nesting Hymenoptera species collected using trap-nests in synanthropic environments in 14 sites, applying a total sampling effort of 255 trap-nest-seasons. The diameter of used trap-nest is given (mm), only the species represented by more than 10 nests are included.

Species	% of sites with the species present	% of trap-nest-seasons with the species present	% of all brood cells	Number of nests	Range of the used diameter	25%-75% quartiles
Pompilidae						
Agenioideus cinctellus	14%	0,8%	0,02%	19	4,1-5,6	4,7-5,2
Auplopus carbonarius	21%	3,1%	0,25%	14	4,4-7,1	5,3-6,6
Dipogon subintermedius	36%	3,1%	0,20%	69	2,7-6,1	3,5-4,5
Vespidae (Eumeninae)						
*Ancistrocerus gazella***	7%	0,8%	0,09%			
*A. antilope***	43%	44,3%	5,25%	437	4,5-8,5	5,8-6,7
A. claripennis	14%	1,2%	0,22%	11	4,4-6,7	4,9-6,2
A. nigricornis	50%	5,9%	0,57%	27	4,0-7,1	4,7-6,2
*A. parietinus***	14%	1,2%	0,09%			
A. parietum	7%	0,4%	0,01%			
*A. trifasciatus***	57%	21,2%	2,16%	301	3,0-8,0	4,3-5,5
*Discoelius dufourii***	14%	5,9%	0,82%	32	3,8-6,1	4,3-5,05
*D. zonalis***	21%	30,6%	3,63%	187	3,4-8,0	5,0-6,0
Euodynerus notatus	21%	4,3%	0,40%	12	4,5-7,7	5,2-6,6
*Symmorphus gracilis***	36%	4,7%	0,72%	260	2,3-6,4	3,7-4,6
*S. allobrogus***	50%	72,2%	53,84%	3154	2,8-8,1	4,5-5,7
S. angustatus	7%	1,6%	0,11%			
*S. bifasciatus***	57%	22,7%	2,78%	65	4,2-8,0	5,3-6,2
*S. crassicornis***	36%	9,0%	1,12%	60	2,2-4,9	3,1-4,0
S. debilitatus	43%	8,2%	0,94%	45	3,2-6,3	4,5-5,5
*S. murarius***	50%	38,0%	7,16%	424	3,9-7,8	5,2-6,3
Crabronidae						
Passaloecus corniger	21%	2,0%	0,26%	34	1,9-5,0	2,3-2,7
P. eremita	7%	0,4%	0,01%			
P. gracilis	14%	0,8%	0,02%			
P. monilicornis	21%	1,2%	0,11%	10	3,0-4,8	3,0-4,0
Pemphredon lugens	7%	0,8%	0,04%			
Psenulus fuscipennis	14%	1,2%	0,57%			
P. pallipes	43%	3,9%	1,06%	21	2,9-5,1	3,4-4,4
Rhopalum clavipes	29%	1,6%	0,08%	19	2,8-5,4	3,2-4,9
Trypoxylon clavicerum	21%	5,9%	0,88%	58	2,0-5,1	2,8-3,6
*T. figulus***	93%	27,5%	9,69%	422	2,9-7,7	4,3-5,5
T. minus	29%	2,7%	0,70%	42	2,8-5,0	3,5-4,3

Species	% of sites with the species present	% of trap-nest-seasons with the species present	% of all brood cells	Number of nests	Range of the used diameter	25%-75% quartiles
Apidae						
Chelostoma florisomne (*Ch. maxillosum**)	43%	3,9%	0,52%	33	2,6-5,2	3,1-4,4
Ch. rapunculi (*Ch. fuliginosum**)	14%	1,6%	0,29%	11	3,0-4,8	3,0-4,0
Heriades truncorum	14%	3,1%	1,14%	35	3,3-5,2	3,8-4,4
Hylaeus miyakei (*H. annulatus**)	7%	0,4%	0,04%			
H. communis	43%	8,2%	1,06%	88	1,0-6,5	3,6-4,6
H. difformis	29%	5,1%	0,89%	21	3,3-6,0	4,3-5,0
H. sinuatus (*H. minutus**)	14%	1,2%	0,08%			
Hoplitis adunca	7%	0,4%	0,02%			
Megachile centuncularis	7%	0,8%	0,15%	24	4,3-7,5	5,25-6,3
M. ligniseca	7%	0,4%	0,01%			
Osmia caerulescens	7%	0,4%	0,02%			
O. leaiana	14%	2,0%	0,49%	14	4,6-6,7	5,5-6,1
O. rufa	43%	9,0%	1,51%	48	4,6-7,3	5,8-6,7

* The name, under which the species was listed in Budrienė *et al*., 2004.
** Species that were selected for more detailed studies of nesting behavior.

We obtained and studied 17112 brood cells of 44 identified cavity-nesting Hymenoptera species: 3 species of Pompilidae, 17 species of Vespidae, 11 species of Crabronidae and 13 species of Apidae. The bees built 1065 (6.2%) of all brood cells found in the trap-nests. Pompilidae and *Trypoxylon*, which hunt spiders, were represented by 6 species and built 2008 (11.7%) of all brood cells. The remaining 14039 (82%) brood cells under study were built by 25 wasp species hunting herbivorous prey. This group was selected for more detailed studies of nesting behavior.

Trypoxylon figulus was the most commonly found species (in 13 of 14 sites), but *Symmorphus allobrogus* was the most abundant species. It was present in a half of all study sites, occurred in more than 70% of all trap-nest-seasons and built more than a half of all brood cells.

A comparison of the range of the nest diameter revealed high flexibility of nesting cavity choice in most of the wasps, however, all species made more than a half of their nests in trap-nests with a range diameter of 1,7 mm (Table 2).

The number of species recorded in the study site had a strict log-linear dependence on sampling effort, measured in the number of trap-nest-seasons — the

regression fit corresponded with the equation $y = (15.5 \pm 1.0)\ log_{10}(x)$ ($R^2 = 0.96$; $p < 0.001$; N=14).

Influence of nesting cavity parameters on nesting behaviour of cavity-nesting solitary wasps

We studied nest structure and the influence of nesting cavity width and length on nesting behavior in 7 wasp species: *Symmorphus allobrogus* (1796 nests), *S. murarius* (270 nests), *S. bifasciatus* (164 nests), *S. gracilis* (42 nests), *S. crassicornis* (50 nests), *Ancistrocerus antilope* (223 nests), and *A. trifasciatus* (220 nests). Additionally, we estimated the influence of nesting cavity width on sex ratio in these and other 6 species: *A. parietinus* (11 nests), *A. gazella* (15 nests), *Discoelius zonalis* (38 nests), *D. dufourii* (10 nests), *Trypoxylon figulus* (39 nests) ir *S. debilitatus* (7 nests).

We hypothesized that wasps have adaptive nesting behaviour and build shorter brood cells in wider nesting cavities. Four hypotheses were tested: the wasp adjusts the length of a brood cell, depending on the nesting cavity width, by **1)** maintaining the volume of the cell more or less constant; **2)** maintaining the area of the longitudinal section of the cell more or less constant; **3)** maintaining the perimeter of the longitudinal section of the cell more or less constant; **4)** the length of brood cell does not depend on the diameter of the nesting cavity.

Most of the species under study demonstrated adaptable nesting behaviour and built shorter brood cells in wider nesting cavities. Our results rejected the assumption that a female wasp simply closes a brood cell after filling it with an appropriate amount of provision - the 1st hypothesis was rejected by all the species (the correlation between cell volume and diameter was strongly significant). Supported hypotheses for species were as follows: the 2nd hypotesis – *A. antilope* (both sexes) and and *S. bifasciatus* (♂); the 3rd - *S. allobrogus* (both sexes), *S. bifasciatus* (♀), *S. murarius* (♂), *A. trifasciatus* (both sexes); the 4th – *S. crassicornis* (both sexes), *S. gracilis* (both sexes), *S. murarius* (♀).Out of seven species under study, only two wasps (*S. crassicornis* and *S. gracilis*) seem to build cells of random length for brooding both sexes in nesting cavities of any diameter. In the other species, the degree of the adaptability of the built cell length to the varying nesting cavity width, applied for the brood of one or both sexes, was statistically significant.

Only in three species (*S. allobrogus*(r=0,39; P<0,001), *S. murarius* (r=0,27, P<0,005) ir *A. antilope* (r=0,20, P<0,01) the number of cells correlated with the nest length.

In three species, the width of nesting cavity affected the size of female progeny: *S.°allobrogus* (r=0,19; P<0,01), *S.°murarius* (r=0,46; P<0,01) and *S.°gracilis* (r=0,79; P<0,001) (Fig. 1), whereas the weight of male progeny did not demonstrated such dependence in any of the species.

All species under the study made more diaphragms than it is needed for cell separation, and the area of diaphragms per progeny weight increased with the nesting cavity width (except *S. crassicornis*).

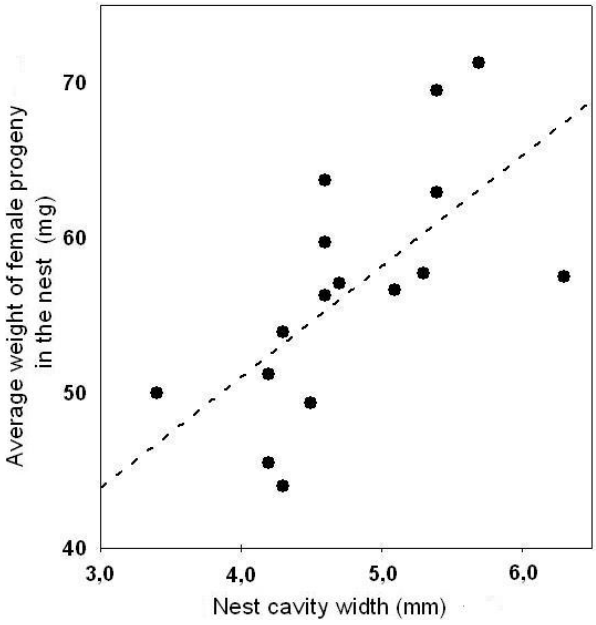

Figure 1. Average weight of female progeny (in the stage of prepupae) in the nests of *S. gracilis*. Regresion fit y=22,5+7,13x

Optimal nesting cavity parameters. We hypothesized that there optimal parameters of nesting cavity, where its volume is exploited most efficiently . We considered the cell density as a parameter indicating the optimal cavity diameter, the distance from the bottom of a nest cavity to the bottom of the first cell as a parameter indicating the optimal depth of the cavity, and the part of cavity length used for cell construction, indicating both optimal depth and the diameter of the nesting cavity. In the available datasets, we found binomial regression fit supporting the hypothesis of optimal parameters of nesting cavity with reference to the above-mentioned nest parameters, except distance from the nest cavity bottom to the bottom of the first cell, for in four species (Table 3).

Table 3. Optimal nest cavity parameters (mm) in consonance with the highest density of cells in nest (cells cm^{-1}) and the biggest usage of nest cavity depth for nest construction (expressed in percent of nest length compared to the length of total length). The values of the highest density of cells and the biggest usage of cavity length are given in brackets.

Species	Opt. diameter (highest density of cells)	Opt. diameter (highest usage of cavity length)	Opt. depth (highest usage of cavity length)
S. allobrogus	6,6 (0,21)	6,4 (44,5%)	179 (43,4%)
S. murarius	6,8 (0,18)	6,4 (47,5%)	
S. crassicornis	6,06 (0,20)	6,04 (47,9%)	
A. antilope			181 (37,7%)

Dependence of a sex ratio on the nest cavity diameter. Since females of trap-nesting solitary Hymenoptera are larger than males, rearing female progeny demands wider cavities. Therefore the diameter of a nesting cavity is one of the factors influencing a sex ratio in the nests of solitary Hymenoptera (e.g. Weaving 1994; Kombein 1967; Oku & Nishida, 1999). A wider trap-nest diameter led to a bigger portion of females in the nests in of 7 of 13 studied species: *S. allobrogus* (r=0,25, p<0,001, N=585), *S. bifasciatus* (r=0,24, p<0,01, N=173), *S. murarius* (r=0,58, p<0,001, N=89), *S. gracilis* (r=0,55, p<0,01, N=21), *S. crassicornis* (r=0,57, p<0,01, N=26), *A. antilope* (r=0,41, p<0,001, N=144), *A. trifasciatus* (r=0,46, p<0,001, N=184) (Fig. 2.), *D. zonalis* (r=0,53, p<0,001, N=37).

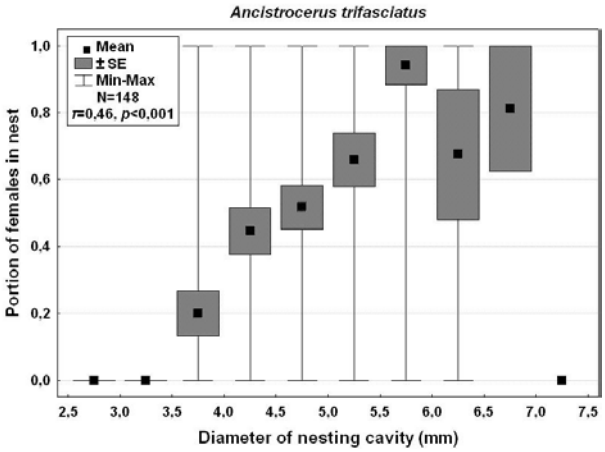

Figure 2. Portion of females in nests of *A. trifasciatus* made in trap-nest with various diameters.

Provisioning behaviour and development of progeny

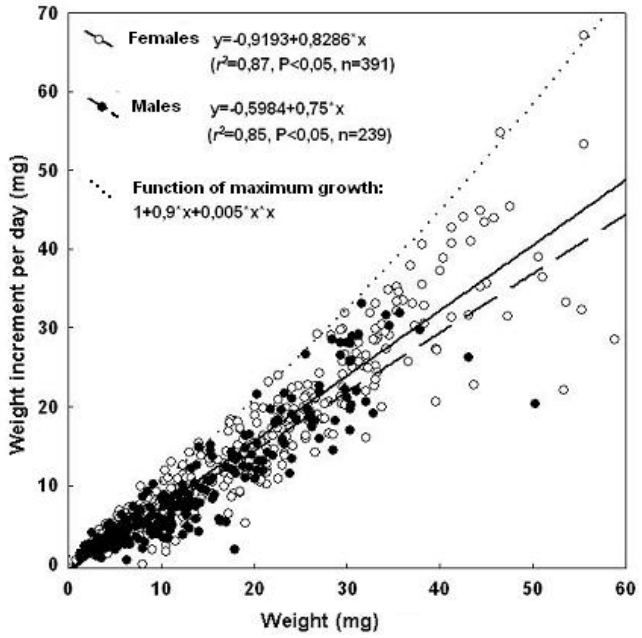

Figure 3. Relationship between larva weight and weight increment per day. Dotted line marks maximum larval

We studied the nest cell content of 5 species. The weight of prepupa was directly proportional to prey weight brought to larva for both sexes of all species, except males of *S. gracilis* (N=7). Regression and determination coefficients defining the relation mentioned above are as follows: *S. allobrogus* (♀ B=0,65, r^2=0,42, N=228; ♂ B=0,76, r^2=0,57, n=138), *S. murarius* (♀ B=0,79, r^2=0,42, N=49; ♂ B=0,64, r^2=0,42, N=41), *S. gracilis* (♀B=0,66, r^2=0,43, N=22), *S. crassicornis* (♀ B=0,88, r^2=0,78, N=15; ♂ B=0,68, r^2=0,47, N=14), *A. antilope* (♀ B=0,78, r^2=0,60, N=27; ♂ B=0,80, r^2=0,64, N=16). Male larvae received from 28% to

43% less of prey than female larvae, but their weight was from 40% to 52% less than the weight of female larvae after consumption of all provision.

Larvae of all studied species have five instars. The estimation of maximal and minimal head width of the larva revealed that it is a good estimator of age, starting from the third instar.

S. allobrogus was selected as a model species for detailed study of progeny development. For the relationship between body weight and weight increment per day, the slopes of male and female regression lines differ significantly (Chow test) - females of a certain weight have bigger weight increment per day than males (Fig. 3). Such difference in weight gain may be conditioned by higher metabolic rates (and associated body weight loss) in males as a smaller sex, especially during the active larval stage, as it was found in other aculeate species (Bosch & Vicens 2002; Cowan 1981; Boomsma & Isaaks 1985 etc.). Having in mind the fact that egg length does not differ between sexes (Mann-Whitney U test), it can be assumed that both sexes have the same starting conditions, so probably a smaller size in males is determined by other factors, not the amount of prey, e.g. adaptive sex-specific patterns of larval resource allocation (Strohm 2000).

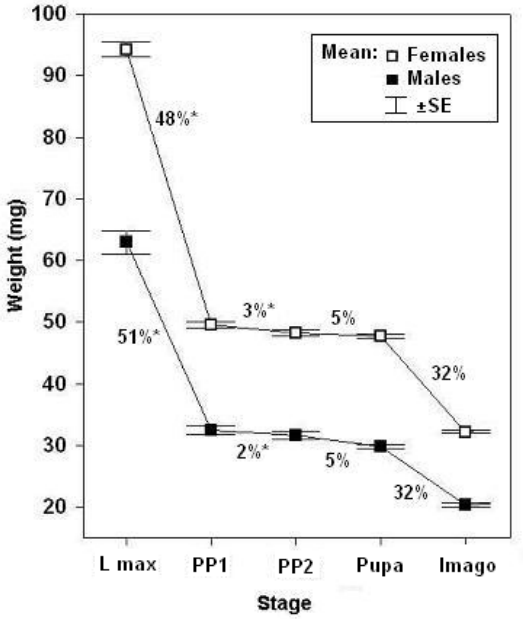

Figure 4. The mean weight of various development stages of *Symmorphus allobrogus* males and females. L max – the maximum weight of larvae, PP1 – prepupae before reactivation, PP2 – prepupae after reactivation, pupa and newly hatched imagoes. Significant differences in weight loss are marked with asterisk.

Weight loss during the development from larva to prepupa was significantly higher in males than in females (Fig. 4) It could be caused not only by higher metabolic rates in males but also by investment of a greater proportion of their larval weight in cocoon construction (Bosch & Vicens 2002), whereas the weight loss during the reactivation period was significantly higher in females. Weight loss during the development to pupa and imago did not differ between sexes.

Effect of prey quantity on the nesting behaviour of *S. allobrogus*

Effect **of prey quantity on the nest structure.** 48 wasp females were kept under permanent regime; 12 females received 5, 15 females – 10 and 10 females - 20 prey individuals per day and made respectively 43, 82 and 29 nests. Twenty one wasp female was kept under shifting regime; 105 nests were made while giving 5, and 98 nests were made while giving 20 prey individuals per day.

Table 4. ANCOVA results: the effect of prey quantity (5, 10, 20 individuals per day) as an independent variable and the mother-wasp's head width, body size and the length of the trap-nest as the covariates on the number of cells per nest (transformed). There was no significant interaction between prey quantity and head width (F=1,57, P=0,21), body weight (F=0,57, P=0,57) and trap-nest length (F=0,85, P=0,43).

	Df	SS	MS	F	P
Head width (beta=-1,62)	1	2,0	2,0	3,2	0,08
Prey quantity	2	19,7	9,9	15,6	<0,001
Error	96	62,2	0,6		
Body weight (beta=-0.01)	1	0,2	0,2	0,4	0,54
Prey quantity	2	23,9	12,0	18,6	<0,001
Error	141	90,5	0,6		
Trap-nest length (beta=0.01)		6,9	6,9	11,5	<0,001
Prey quantity	2	13,4	6,7	11,2	<0,001
Error	150	89,9	0,6		

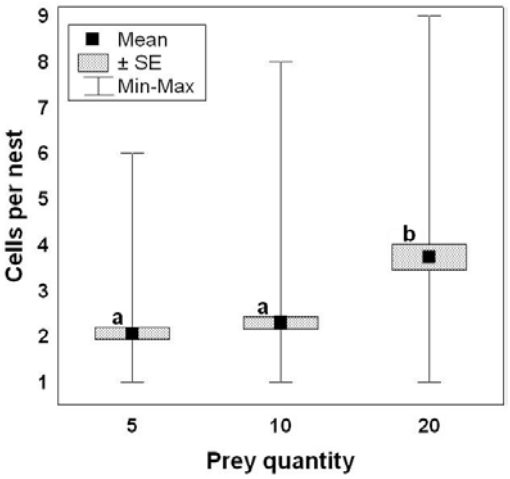

Figure 5. The number of cells in the nests made under permanent regime. Different letters indicate significant differences between groups of prey quantity (Scheffé test).

Wasp females responded to a higher amount of available prey by building more cells per nest in permanent regime (Tab. 4, Fig. 5) as well as in shifting regime (F=25,0; P<0,001; $NumDf$=1; $DenDf$=107). The only covariate out of three, the nesting cavity length, had a significant positive influence on the cell number per nest (Tab. 4). Number of diaphragms fall on a cell did not differ between groups of prey quantity (Kruskal-Wallis test).

Effect of female age on its nest structure. Older wasps made significantly fewer cells per nest, with exception of the dataset of nests made under shifting regime, 5 prey specimens per day). Spearman correlation coefficients were as follows: permanent regime, 5 preys : r=-0,42, P=0,003, N=49; 10 preys : r=-0,33, P=0,003, N=82; 20 prey: r=0,58, P=0,000, N=40; shifting regime, 20 preys: r=-0,21; P<0,05; N=97.

Effect of prey quantity on provisioning behaviour and the sex ratio. Some cells were provisioned with the bigger weight of prey while receiving 20 and 10 preys per day, but this did not influence the size of progeny: the body weight and head width of larva measured individually as well as the mean parameters of each sex in the nest did not differ between the prey quantity groups.

The proportion of female progeny in a nest depended on the number of cells – the larger number of cells, the smaller portion of females was present in the nests made

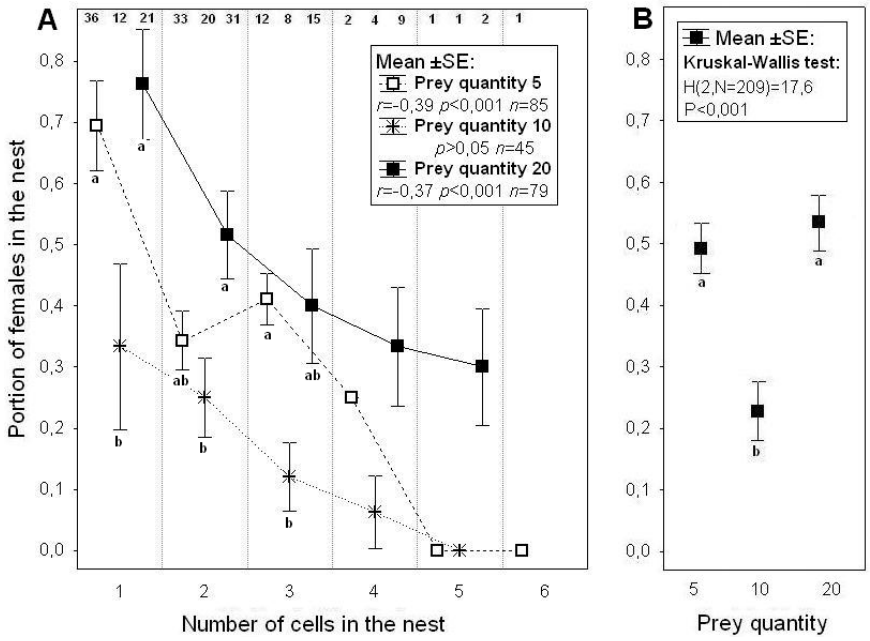

Figure 6. The portion of females in nests containing 1-6 cells while receiving 5, 10 or 20 prey individuals per day (A) (sample sizes are given in the upper part of the graph) and the mean portion of females in all the nests (regardless of the number of cells in the nest) while receiving a different amount of prey. Different letters indicate significant differences between groups of prey quantity (Kruskal-Wallis, Mann-Whitney U tests; nests containing more than 3 cells were not tested due to a small sample size).

under regimes of 5 and 20 prey items per day (Fig. 6 A). On average, the portion of females was approximately equal under regimes of 5 and 20 prey items per day (0,49±0,04 and 0,53±0,05, respectively), whereas the wasps that were provided with 10 prey items per day produced distinctly fewer females (0,23±0,05) (Fig. 6 B). The proportion of female progeny made by each wasp in comparison with the total of its progeny was slightly smaller, but had the same tendency: in the group of prey quantity 10, the share of females was the smallest - female progeny comprised 18,6 % of the total progeny, respectively in groups of prey quantity 5 and 20, female progeny comprised 37,7 % and 44,6 % of the total progeny.

Ageing had a negative effect on provision weight per cell, progeny size and the share of female progeny per nest, although it was not always significant. Probably due to an insufficient sample size, we did not succeed in assessing the effect of the wasp size on these nesting parameters.

Wasps given the maximal prey amount per day received prey items four times as much as wasps given the minimal prey amount, but the overall number of cells made during the lifelong experiment was approximately only twice larger than in the group of the minimal prey amount (Fig. 7). These results confirm that oocyte production may be the factor limiting reproduction success under conditions of high prey availability (Rosenheim *et al*. 1994, Minckley *et al*. 1994).

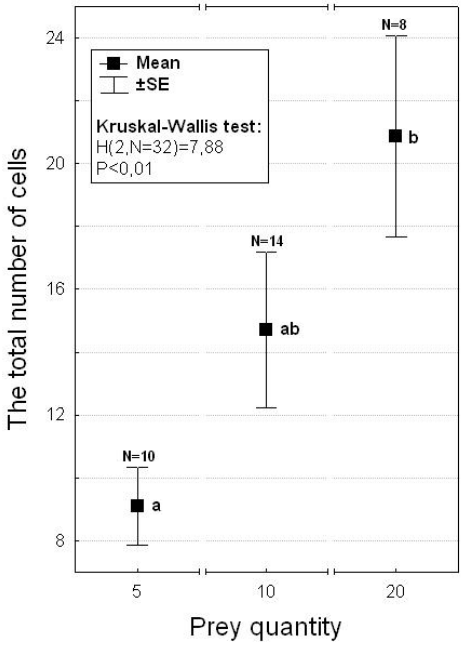

Figure 7. The total number of cells made by wasps while receiving a different amount of prey per day. Different letters indicate significant differences between groups of prey quantity (Kruskal-Wallis (the results are presented in the legend), Mann-Whitney U tests: Z=-2,89; P<0,01).

Nesting behviour of virgin females. Out of 14 virgin females, 10 were kept under permanent prey provision regime (4 females received 5, 1 female – 10 and 5 females - 20 prey individuals per day and built 13, 3 and 27 nests, respectively), and 4 females were kept under shifting regime (12 nests were built while giving 5, and 11 nests were made while giving 20 prey individuals per day).

The results were inconsistent: the number of cells in the nests made under permanent regime did not differ between groups of prey quantity 5 and 20 (the group of prey quantity 10 was not included in the analysis because of single observation), whereas the wasps under shifting regime made significantly fewer cells per nest while receiving 5 prey items per day than receiving 20 prey items per day. The additional research is needed for unambiguous conclusion regarding virgin females.

Defended conclusions

1. The wasp communities inhabiting reed trap-nests in synanthropic sites in Lithuania include up to 44 species. The number of species recorded in the site depends on sampling effort. Hymenoptera inhabiting a trap-nest are flexible in choice of the nesting cavity diameter.
2. Most of the species under study have adaptable nesting behaviour and build shorter brood cells in wider nesting cavities. The size of female progeny and the sex ratio in a nest are influenced by the width of a nesting cavity. In some cases, more cells may be built in longer cavities. The nesting cavity may have optimal parameters for species: the dependence of its usage efficiency parameters on depth and the diameter of the nesting cavity can have a convex pattern.
3. The wasp provides a different amount of prey to the progeny of different sex. The larvae of the 3rd, 4th and 5th instar can be reliably distinguished by their head width. The assimilation of food and weight loss during the development of full-grown larva to pupa differ between *S. allobrogus* individuals of different sex.
4. Wasps build more cells per nest and provision cells with a slightly higher amount of prey, when prey availability is higher. However, the progeny size, the sex ratio and number of diaphragms do not depend on the available amount of prey.
5. The number of cells per nest, progeny weight and the proportion of female progeny decline with aging of the wasp female.

THE LIST OF PUBLICATIONS AND CONFERENCE ABSTRACTS CONTAINING MATERIALS OF THE THESIS

Budrienė, A., Budrys, E. & Nevronytė, Ž. 2004. Solitary Hymenotpera Aculeata inhabiting trap-nests in Lithuania: Nesting cavity choice and niche overlap. *Latvijas Entomologs 41*, 19-31.

Budrys, E., Budrienė, A., Nevronytė, Ž. 2009. Dependence of brood cell length on nesting cavity width in xylicolous solitary wasps *Ancistrocerus* and *Symmorphus* (Hymenoptera: Vespidae). *Acta Zoologica Lituanica* 19 (4), (accepted).

Budrys, E., Budrienė, A., Nevronytė, Ž. 2009. Check-list of Eumeninae wasps (Hymenoptera: Vespidae) collected in Lithuania using trap-nests. *New and Rare for Lithuania Insect Species. Record and Descriptions.* 21 (accepted).

Nevronytė Ž. 2005. Influence of the nest cavity parameters and cleptoparasites on reproductive behaviour of the solitary wasps. *Biodiversity, Molecular Ecology and Toxicology. Palanga, Lithuania, 29-30 November, 2005*, 46 psl.