

## The Significance of the Vestibular Cell in Trap Nesting Wasps (Hymenoptera: Crabronidae): Does its Presence Reduce Mortality?

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*In a study carried out with the trap-nesting solitary wasp Trypoxylon attenuatum (Hymenoptera: Crabronidae) in 2000, we analysed the significance of different nest characteristics, like the vestibular cell and empty intercalary cells, and its role in the reduction of mortality. Analysis of the presence of the vestibular cell suggested that it represents a nest completion structure, whose presence reduces mortality in the initial stages (MI) but does not affect that due to chrysidid parasitoids (MP). The length of such vestibular cell was found to depend mainly on the space between the last provisioned cell and the exterior, pointing to a possible role in the reduction of the remaining space that could be used by other females to establish a nest exterior to the completed one. The empty intercalary cells were smaller than the vestibular cells, did not affect MI or MP mortality rates in the nest, and exhibited an “aggregated” pattern of occurrence. This suggests that they could be the result of “abnormal” behaviour by the female owning the nest. The total mortality rate decreased with the increase in the number of cells in the nests, also being greater in nests established at low heights above the ground. MI was lower in the innermost cells, while MP increased towards the interior and in the nests with more cells. The distribution in the nests of cells parasitized by Trichrysis cyanea (the main natural enemy found in the wasp population studied) reflects an “aggregated” pattern, suggesting repeated attacks against certain nests.*

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**KEY WORDS:** vestibular cell; intercalary cell; mortality; *Trypoxylon*; Crabronidae.

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## INTRODUCTION

The females of many hymenopteran Crabronidae, Vespidae and Apidae establish their nests in the stems of plants, excavating the plant tissue or using galleries and cavities previously constructed by other insects. These species are known as rubicolous and, in many cases, the nests comprise linear series of cells arranged one after the other and separated by septa (Fig. 1). Different authors have described the presence of a “vestibular cell” (VC) (an empty “cell” situated in the outermost part of the nest) in the nests of several species of Pompilidae (Weaving, 1995), Eumeninae (Jayasingh and Taffe, 1982; Weaving, 1995; Assis and Camillo, 1997; Sears *et al.*, 2001), Apidae (Malyshev, 1936; Krombein, 1967; Rust, 1986; Seidelmann, 1999; Vieira de Jesús and Garófalo, 2000; Camillo, 2005) and Crabronidae (Krombein, 1967; Coville and Coville, 1980; Coville and Griswold, 1983; Camillo *et al.*, 1993, 1994; García and Adis, 1995; Assis and Camillo, 1997; Matthews, 2000), and that structure has been cited as being present frequently (Asís *et al.*, 1994) or occasionally (Danks, 1970) in *Trypoxylon attenuatum*. The meaning of that structure remains to be elucidated. Although it has been proposed that it could play a defensive role against the action of certain natural enemies (Krombein, 1967; Coville and Coville, 1980; Coville, 1982; Coville and Griswold, 1984), no conclusive data have emerged to confirm its function. García and Adis (1995) and Weaving (1995) failed to find a relationship between the presence of VC and a reduction in parasitism. Seidelmann (1999) reported a higher presence of VC in nests established in exposed environments, the length of that structure also being significantly smaller in nests placed in protected areas. However, the parasitism was not reduced with the presence of VC. That author rejected that VC might have a function in parasite defence, proposing a protective role against adverse environmental conditions for large VCs of exposed nests, and an avoidance of the risk of provisioning the exterior cell (by leaving it empty) in the presence of different natural enemies. Also, the small VCs observed in nests located in protected areas would represent an end space, insufficient to harbour a provisioned cell.

Empty intercalary cells (EIC) (Fig. 1) have also been found in the nests of many rubicolous species, although their presence is less frequent than that of VC (Malyshev, 1936; Krombein, 1967; Jayasingh and Taffe, 1982;



**Fig. 1.** Idealised nest of *T. attenuatum* (the opening is at the right): VC, vestibular cell; EIC, empty intercalary cell; C1-C6, cells 1–6 (C1 has M1; C2 and C3 include male cocoons; C4 harbours a chrysidid cocoon (MP), and C5 and C6 harbour female cocoons).

Assis and Camillo, 1997; Sears *et al.*, 2001). Krombein (1967) suggested that this would reflect a relict behaviour, deriving from the establishment of series of *provisioned cell-vestibular cell* in longer cavities, in order to optimise the use of space in such cavities. Malyshev (1936) proposed that they could reflect a possible aberrant behaviour of older-aged individuals or an exhaustion of the egg supply. Evans and West Eberhard (1970) suggested a role in the reduction of parasitism, and Tepedino *et al.* (1979), by means of simulation, demonstrated a possible reduction in it, although the model developed by those authors was only applicable to species that build nests with mud, with the cells grouped and with a direct exit to the exterior (e.g. *Sceliphron*).

In the present study, we attempt to analyse which factors could induce the establishment of a vestibular cell and empty intercalary cells in the nests of rubicolous species, specifically testing whether their presence involves a reduction in mortality (as being suggested in several studies) and whether they could be related structures, with a similar function. We also analyse, from a multivariate perspective, the possible influence of different characteristics of trap nests (diameter, available space, number of cells) and of their location (nature of the substrate, and height above ground) on the mortality rate (both total and partial) in order to explain the differences observed in the mortalities affecting different populations of wasps.

As a model, we used a population of the crabronid wasp *Trypoxylon attenuatum* (Smith, 1851) in the central-western area of the Iberian Peninsula. *T. attenuatum* is a species with a Palaearctic distribution, common in southern Europe (Bohart and Menke, 1976; Antropov, 2001). The females establish their nests in hollow plant stems (often in cavities excavated by other aculeates), with 1 to 11 cells (separated by mud partitions) in linear succession, which they provision with 3–27 spiders (Hamm and Richards, 1930; Freeman, 1938; Grandi, 1961; Danks, 1970; Abraham, 1982; Asís *et al.*, 1994; Tormos *et al.*, 2005). The distribution of sexes inside the nests is not random: the cells with females are found in the innermost, while the outermost cells usually harbour males (Asís *et al.*, 1994; Tormos *et al.*, 2005). Some nests have an empty, unprovisioned “vestibular cell” (VC) in the outermost part of the cavity. Additionally, the presence of “empty intercalary cells” (EIC) has been reported; these are arranged between the series of provisioned cells (Danks, 1970; Asís *et al.*, 1994) (Fig. 1).

## MATERIALS AND METHODS

The study was carried out at two localities (Ejeme and Villagonzalo de Tormes) in the province of Salamanca (western Spain), separated by some 20 km. The type of habitat is the same at both sites: a gallery forest on the

banks of the river Tormes with a predominance of willow, black poplar, ash and wild rose. In April 2000, 1330 trap nests, made of reed stems (*Phragmites australis*) of varying length (32–245 mm), and diameter (2–4 mm), and grouped in bundles of 5 stems, were placed in the field. These bundles were fixed, with adhesive tape, on four different substrates (*Alnus glutinosa* (L.) Gaerthn, *Fraxinus angustifolia* Vahl., *Rosa canina* L. and *Salix atrocinerea* Broth.), at 3 different heights above the ground (low = 90 cm; medium = 140 cm; and high = 190 cm). The bundles remained in the field until the beginning of October 2000, when they were collected and taken to the laboratory. The stems were sectioned longitudinally to study the structure of the nests (mud partitions, number and dimensions of the provisioned cells, presence of VC and EICs) and the contents of each cell. During the winter, the larvae and prepupae were placed in glass vials at 5–8°C, rearing them at environmental temperature and photoperiod in the following spring to elicit the emergence of the adults.

Most nests of the genus *Trypoxylon* found in the study belonged to *T. attenuatum*, although some had been established by *T. figulus* (22 of the 159 nests from which adults were obtained). Since it was not possible to separate the nests of both species attending only to their structure, in the analysis only the nests in which at least one adult of *T. attenuatum* was present were included. Of this latter species, 137 nests were studied, containing 507 provisioned cells.

To analyse mortality, we considered the number of cells from which adults emerged; the number of cells parasitized (MP); the number of cells with uneaten prey (owing to the absence of oviposition, attack by fungi, or possible failed parasitism at early stages) (M1); and the number of cells with failed larvae or pupae (M2).

Of the 137 nests studied, only in 42 (30.7%) did all the progeny reach the adult stage (i.e., mortality rate = 0). These nests showed IFOM (inner females and outer males), AM (all males), and AF (all females) sex sequences (see Oku and Nishida, 2001), no IMOF (inner males and outer females) or IRR (irregular distribution) (see Introduction section) patterns being observed. Because the presence of IMOF or IRR nests was zero, we estimated, in the nests where this was possible, the sex of dead individuals as a function of the position occupied in the cell. Sex estimation was performed in such a way that the cells located outside a cell from which a male had been obtained were considered male cells, and those further inside a cell containing a female as female cells (see Oku and Nishida, 2001). After this estimation, for 82 nests 80 had AM, AF or IFOM sequences, only one being IMOF and the other IRR; the number of nests found in the IFOM, AM and AF types in the sample of 82 nests did not differ significantly

from those found in the sample of 42 nests (Chi-square test:  $\chi^2_2 = 2.63$ ,  $p = 0.268$ ).

The influence of different variables –stem diameter, available space, locality, height, presence or not of VC and number of cells occupied- on the mortality rate was analysed with a general linear model. The mortality rate ( $10^{\wedge}$ mortality rate), the number of cells occupied (ln cell number) and the space available (square-root available space) were transformed to achieve normality. To analyse the influence of the different variables on the various mortality classes (M1, M2 and MP), and the mortality in the cells as a function of their position (cell 1 being the outer one), Chi-square tests were employed. The presence of vestibular cell and empty intercalary cells, together with the M1 mortality rate, was analysed by logistic regressions. All analyses were done with XLStat 6.0 and Minitab 13.0.

## RESULTS

### Mortality Analysis

The mortality rate calculated for the nests from which at least one adult of *T. attenuatum* had been obtained was 36.1%. However, the real mortality rate must be higher and could reach 50%, which was the value obtained on including the nests from which no adults were obtained (i.e., nests with a 100% mortality of cells). The mortality recorded for the different classes (M1, M2 and MP) was not different for both data sets (Chi-square test,  $\chi^2_2 = 1.91$ ,  $p = 0.385$ ). Table I shows the mortality values obtained in the study as a function of the different variables analysed. A general linear model analysis revealed that only the height above ground at which the stem had been placed and the number of cells occupied (and its quadratic term) entered the minimal adequate model ( $F_{4,132} = 6.42$ ,  $p < 0.0001$ ,  $R^2_{\text{adj}} = 0.138$ ); the remaining explanatory variables introduced in the preliminary model (locality, substrate, length of the cavity, presence of a vestibular cell, presence of empty intercalary cells, and cavity diameter) did not significantly affect the mortality rate obtained for the nests. The mortality rate was lower in the nests situated at 190 cm and greater in those at a height of 90 cm ( $F_{2,132} = 4.44$ ,  $p = 0.014$ ). The number of occupied cells and its quadratic term also entered in the minimal adequate model ( $F_{1,132} = 10.79$ ,  $p = 0.001$  for  $\ln(\text{number of cells occupied})$ ;  $F_{1,132} = 5.26$ ,  $p = 0.023$  for  $\ln(\text{number of cells occupied})^2$ ), their coefficients (2.02 for  $\ln(\text{number of cells occupied})$  and  $-0.56$  for  $\ln(\text{number of cells occupied})^2$ ) revealing a non-linear relationship, with an increase in the mortality rate

**Table I.** Contents of the nests of *Trypoxylon attenuatum* (for which at least one adult was obtained) as a function of locality, substrate and height above the ground, presence or not of a vestibular cell in the nest, and position of the cell (cell 1 is the outermost one)

	Adults	MT	M1	M2	MP
Locality					
Ejeme	245	135	31	89	15
Villagonzalo	79	48	12	28	8
Substrate					
Alnus	22	7	0	6	1
Fraxinus	38	22	5	14	3
Rubus	31	14	6	8	0
Salix	233	140	32	89	19
Height					
90 cm	48	47	5	42	0
140 cm	183	106	29	56	21
190 cm	93	30	9	19	2
VC					
Absent	75	46	18	28	0
Present	249	137	25	89	23
Cell position					
1	90	47	14	32	1
2	82	44	14	25	5
3	58	26	7	13	6
4, 5	50	34	6	27	1
6–10	44	32	2	20	10
Total	324	183	43	117	23

Note. MT (total mortality), M1 (absence of oviposition, egg mortality, fungi attack in early stages), M2 (mortality in the larval and pupa stages), MP (mortality due to parasitoids). VC (vestibular cell).

when the number of cells was low and a decrease in the rate for nests with 6 or more cells.

The partial mortalities (M1, M2 and MP) were similar in the nests collected at both study sites (Chi-square test,  $\chi^2_2 = 1.25$ ,  $p = 0.535$ ), and neither were any significant differences found as a function of the substrate used to place the nests (Chi-square test,  $\chi^2_6 = 6.44$ ,  $p = 0.376$ ). Nest height influenced both the total mortality (lower mortality in nests located higher above the ground) and the partial mortalities: MP was greater in the nests situated at medium height while M1 decreased in those situated lower down (Table II).

Total mortality did not vary significantly as a function of the position of the cell in the nest, although this variable did modify the partial mortalities: mortality due to parasitoids was greater in the cells located towards the beginning of the nest and less in the outer cell, whereas M1 was significantly lower in the innermost (Chi-square test,  $\chi^2_8 = 27.12$ ,  $p < 0.001$ ) (Table II).

**Table II.** Variables that significantly affect mortality, total or partial, of the nests of *T. attenuatum*: height above ground; presence or not of a vestibular cell in the nest, and cell position in the nest (cell 1 is the outermost one). For each class of mortality, the significance of the variable analysed and its direction are shown. For each variable and analysis, the results of the Chi-squared test and associated probability are indicated

	M1	M2	MP	MT
Height				
90 cm	(-) <sup>***</sup>	(+) <sup>***</sup>	(-) <sup>***</sup>	(+) <sup>***</sup>
140 cm	NS	(-) <sup>***</sup>	(+) <sup>***</sup>	NS
190 cm	NS	NS	NS	(-) <sup>***</sup>
Chi-square test		$\chi^2_4 = 22.48, p < 0.001$		$\chi^2_2 = 14.72, p < 0.001$
VC				
Absent	(+) <sup>***</sup>	NS	(-) <sup>***</sup>	NS
Present	(-) <sup>***</sup>	NS	(+) <sup>***</sup>	NS
Chi-square test		$\chi^2_2 = 14.20, p < 0.001$		$\chi^2_1 = 0.16, p = 0.692$
Cell position				
1	NS	NS	(-) <sup>**</sup>	NS
2	NS	NS	NS	NS
3	NS	NS	NS	NS
4, 5	NS	(+) <sup>*</sup>	NS	NS
6-10	(-) <sup>**</sup>	NS	(+) <sup>***</sup>	NS
Chi-square test		$\chi^2_8 = 27.12, p < 0.001$		$\chi^2_4 = 3.12, p = 0.538$

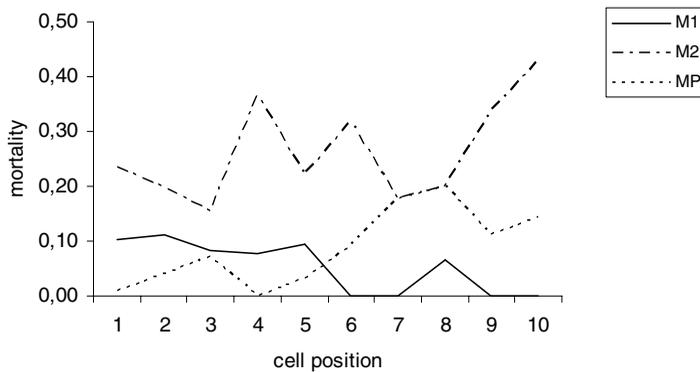
Note. MT (total mortality), M1 (absence of oviposition, egg mortality, fungi attack in early stages), M2 (mortality in larval and pupa stages). VC (vestibular cell). NS (not significant), \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . + (greater mortality) - (less mortality).

A regression analysis of the M1, M2 and MP mortalities as a function of cell position in the nest revealed a decrease in M1 ( $rate\ of\ M1 = 0.124 - 0.013 * cell\ position; F_{1,8} = 15.49, p = 0.004, R^2_{adj} = 0.617$ ) and an increase in MP ( $rate\ of\ MP = -0.011 + 0.018 * cell\ position; F_{1,8} = 12.01,$

**Table III.** Estimation parameters for the logistic regression models of the presence of vestibular cell and empty intercalary cells in the nests studied and of the probability of establishment of the different types of nests. Only the statistically significant factors are included

Parameter	Estimates
Presence of VC	
Intercept (NS)	0.693
Nest type: AM (-)	0.795
Nest type: IFOM (NS)	0.061
Nest type: AF (**)	-0.856
Presence of EIC	
Intercept (***)	-6.782
Available space (sqr) (***)	0.609
n. cells occupied (ln) (**)	-1.756

Note. VC (vestibular cell), EIC (empty intercalary cells). NS (not significant), \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . AM (all male nests), AF (all female nests), IFOM (inner females outer males nests).



**Fig. 2.** Mortality observed in *T. attenuatum* as a function of the position of the cell (cell 1 is the outermost one). M1, mortality in initial stages or absence of oviposition; M2 mortality in larvae or pupae; MP mortality due to natural enemies.

$p = 0.009$ ,  $R_{\text{adj}}^2 = 0.550$ ) towards the inner cells, while M2 did not follow a definite trend ( $F_{1,8} = 2.54$ ,  $p = 0.150$ ) (Fig. 2). Additionally, a logistic regression analysis revealed an increase in the probability of MP as a function of the number of cells in the nest ( $\chi_1^2 = 4.94$ ,  $p = 0.026$ ,  $R^2$  (McFadden) = 0.055).

The rate of M1 was analysed with the sub-sample of 80 nests for which the sequence of sexes was known (see Materials and Methods). A GLM analysis of the M1 rate revealed that the only significant factor was the presence of a vestibular cell, which elicited a decrease in that mortality rate ( $F_{1,78} = 10.07$ ,  $p = 0.002$ ). Additionally, factors such as the number of cells, the type of nest (AM, AF or IFOM), the available space, nest height or diameter, did not have a significant influence in this respect.

Only 23 of the 507 cells studied had been parasitized (4.54%), all of them by Chrysididae. Most of the attacks were due to *Trichrysis cyanea* (20 cells), while *Chrysidea pumila* (Klug) appeared in only one of the cells (in another two, the identity of the parasitoid could not be determined).

*T. cyanea* was found in 11 nests (8.03%), in which it parasitized 33.3% of the cells (20/60). The fitting of the presence of the parasite in the nests to a negative binomial distribution ( $k = 0.107$ ;  $\chi_2^2 = 0.48$ ,  $p = 0.785$ ) suggests an aggregated pattern. The sex ratio (males/males + females) found for the parasitoid was 0.824, significantly different from 0.5 (binomial test,  $z = 2.43$ ,  $p < 0.01$ ) and strongly biased towards males.

### Vestibular Cell (VC)

74.5% of the nests studied had a vestibular cell. The presence of VC, although it did not influence the total mortality rate, produced variations in the mortality rates affecting the different developmental stages: M1 was significantly reduced in the cells of nests with VC, whereas the mortality brought about by parasitoids (MP) was greater in this type of nests (Chi-square test,  $\chi^2_2 = 14.20$ ,  $p < 0.001$ ); M2 was not modified by the presence of a VC (Table II).

A logistic regression analysis revealed that only the type of nest (AM, AF or IFOM) modified the probability of the appearance of VC ( $\chi^2_2 = 9.44$ ,  $p = 0.009$ ,  $R^2(\text{McFadden}) = 0.102$ ), whereas the available space, the number of cells, and the height of the nest above the ground did not show any significant influence. The AM and IFOM nests showed a greater probability of having VC than the AF nests (Table III). It should also be noted that all the nests in which MP was observed also had VC.

The mean number of cells in the nests with VC (mean =  $3.78 \pm 2.56$ ,  $n = 102$ ) was greater than that of nests without VC (mean =  $3.46 \pm 2.12$ ,  $n = 35$ ), although the differences were not significant (Student's  $t$  test,  $t_{135} = 0.66$ ,  $p = 0.255$ ).

The length of the VC varied between 3–91 mm. A GLM analysis revealed that the most significant factor in VC length was the separation of the last provisioned cell (i.e., the free space between the last mud partition and the exterior, before the establishment of the VC) ( $F_{1,52} = 16.22$ ,  $p < 0.001$ ); although to a lesser extent, the final available space also had an effect ( $F_{1,52} = 5.53$ ,  $p = 0.023$ ). The number of cells, the type of nest (AM, AF or IFOM) and the height of the nest above ground did not enter the minimum adequate model, although the last two variables were close to the level of significance of 0.05 ( $F_{2,52} = 2.55$ ,  $p = 0.088$  for *nest type*;  $F_{2,52} = 2.99$ ,  $p = 0.059$  for *height*) (Table IV).

The hypotheses that VCs might be cells destined for harbouring progeny but that, for unknown reasons, have not been provisioned (VCs would thus be similar in length to provisioned cells) or that they might correspond to an space remaining at the end of the nest that was insufficient to harbour a provisioned cell (in which case the length of the VC would be less than that of these cells) were ruled out after comparing length cell: VCs were much longer (mean =  $24.07 \pm 15.98$ ,  $n = 102$ ) than the provisioned cells (mean =  $13.91 \pm 4.51$ ,  $n = 507$ ) or the empty intercalary cells (EIC) (mean =  $13.12 \pm 6.04$ ,  $n = 44$ ) (ANOVA,  $F_{2,651} = 77.32$ ,  $p < 0.0001$ ). The *a posteriori* Tukey test revealed significant differences between the VCs and the other two types of cell (although not between the occupied cells and the EICs). Furthermore, the VCs were also longer than

**Table IV.** Coefficients and associated probability for the terms included in the GLM analysis of the length of the vestibular cell. Although not significant, the type of nest and height above ground of the stem are included ( $F_{6,52} = 8.63, p < 0.0001, R_{\text{adj}}^2 = 0.441$ )

Parameter	Coefficient	<i>P</i>
Intercept	-28.86	0.007
Available space (sqr)	2.396	0.023
Separation 1st cell (sqr)	3.721	0.000
Nest type: AF	-6.667	0.033
Nest type: IFOM	2.899	NS
Height: 190 cm	-6.228	0.018
Height: 90 cm	6.696	NS

Note: AF (all female nests), IFOM (inner females outer males nests).

the cells from which males had been obtained (established in the outer part of the nest by the females) (mean =  $13.69 \pm 4.50, n = 165$ ) (Student's *t* test (Satterthwaite's method for non-equal variances),  $t_{110} = 6.37, p < 0.0001$ ).

Since the GLM carried out with the length of the VCs reflected a smaller size of this structure in the AF nests (see Table IV), we compared the size of the VCs in the different nest types. In AF nests, the length of the VC and those of the provisioned cells were similar (Mann-Whitney *U* test,  $z = -0.3, p = 0.77, N_1 = 507, N_2 = 8$ ), while in AM and IFOM nests, VC length was significantly greater than those of the provisioned cells (Mann-Whitney *U* test,  $z = -2.61, p < 0.01, N_1 = 507, N_2 = 31$ , for the AM nests; Mann-Whitney *U* test,  $z = -4.59, p < 0.001, N_1 = 507, N_2 = 20$ , for the IFOM nests).

### Empty Intercalary Cells (EIC)

Of the 137 nests studied, 23 (16.8%) had empty intercalary cells (EICs). In the nests where they were present, they accounted for an important fraction of the total number of cells: 40.7%. This high value suggests an aggregated distribution of EICs, seen upon analysing the fitting of the data observed at a negative binomial distribution ( $k = 0.1792; \chi_4^2 = 2.56, p = 0.633$ ). As reported above, the Tukey *a posteriori* test did not reveal significant differences between the length of the EICs and that of the provisioned cells, but did show that the EICs were significantly shorter than the VCs. The mean number of occupied cells in the nests without EICs ( $3.89 \pm 2.55, n = 114$ ) was greater than in the nests with EICs ( $2.78 \pm 1.65, n = 23$ ) (Student's *t* test,  $t_{135} = 1.99, p = 0.024$ ), although the overall mean number of cells for both groups of nests (nests without EIC:  $3.89 \pm 2.55, n = 114$ ; nests with EIC:  $4.70 \pm 2.16, n = 23$ ) was similar (Student's *t* test,  $t_{135} = 1.42, p = 0.079$ ).

A logistic regression analysis showed that only the space available in the cavity and the number of occupied cells significantly modified the probability of EICs appearing, whereas nest height, the presence of a VC or the existence of “anomalous” cells (such as parasitized cells or cells with M1 mortality) did not show significant influences ( $\chi^2_7 = 24.62$ ,  $p < 0.001$ ,  $R^2$  (McFadden) = 0.199). The probability of there being EICs in a nest was increased in nests with greater available space and decreased when the number of occupied cells was greater (Table III). Analysis of the sub-sample of 80 nests for which the sex sequence was known reflected the same result and did not reveal any influence of the type of nest involved (AM, AF or IFOM) with respect to the presence of EICs. No significant differences were observed between the different classes of mortality (M1, M2 and MP) as a function of the presence or absence of EICs ( $\chi^2_2 = 2.95$ ,  $p = 0.228$ ).

## DISCUSSION

### Mortality

Several authors have reported the interpopulation differences in the mortality affecting given species of Crabronid wasps, both those that nest in the ground (Hook and Matthews, 1980; Spofford *et al.*, 1986; Evans and O'Neill, 1988) and those that build their nests in cavities (Coville and Coville, 1980; Weaving, 1995; Tormos *et al.*, 2005). These differences reflect the variations in the selective pressures that act on the different wasp populations, both as regards biotic factors (e.g., communities of natural enemies present in the area) and abiotic issues. The present study demonstrates, for example, the influence of height above ground in the location of the nests in the total mortality: the higher nests underwent less mortality. However, the analysis of partial mortalities revealed a greater pressure by the natural enemies at medium heights (perhaps as a result of a preferential search at that height interval by the main natural enemy of this species, *Trichrysis cyanea*), and a reduction in M1 mortality at the low height. These variations suggest that changes in the environmental conditions or the presence of different communities of natural enemies would lead to variations in mortality; the differences in MT, MP and M1 are a reflection of the complex balance that exists in any animal community and of its close relationship with external factors. In this sense, an analysis of the mortality values found for different populations of *Trypoxylon attenuatum* (Danks, 1970; Asís *et al.*, 1994; Tormos *et al.*, 2005) reveals small differences in the overall mortality rates (between 33.2%–44.0%) that are not statistically different (Chi-square test:  $\chi^2_3 = 6.94$ ,  $p = 0.074$ ), although the mortality rates due to parasitoidism differ (between 2.4%–

20.0%) from some populations to others (Chi-square test:  $\chi^2_3 = 48.73$ ,  $p < 0.0001$ ).

Analysis of the partial mortality rates reveals differences as a function of the position of the cells. M1 tends to become reduced towards the interior of the nests, suggesting that a greater isolation from the exterior could lead to a decrease in this class of mortality. The fact that in the nests with VC, M1 was also reduced, supports this notion; additionally, the nests situated at a lower height (and hence probably less exposed) also showed a lower M1. Additionally, MP increased towards the interior of the nest, and nests with more cells had a greater probability of attacks by parasitoids. It should be noted that the chrysidids were the only natural enemies of *T. attenuatum* in the study area. These parasitoids access the “active” cells of the host (i.e., those that are being provisioned), taking advantage of the absence of the female, before the cell is sealed with mud (Krombein, 1967; Sears *et al.*, 2001). In *Argochrysis armilla* Bohart, Rosenheim (1987) demonstrated the ability of some chrysidids to “memorise” the position of the nests of their hosts, returning to them on repeated occasions to parasitize the active cell. In the case of *Trichrysis cyanea* having this ability, it would be logical to expect a greater probability of parasitoidism in nests with a greater number of cells, since the probabilities of locating a nest are greater if this remains active for a longer time (as would be the case of nests with a greater number of cells). Also, the fact that the cells attacked by the chrysidid showed an aggregated pattern (that is, that there tends to be a greater number of nests with several parasitized cells than would be expected by chance) from a random distribution as seen from the fitting of the data to a negative binomial distribution, supports this notion. Nevertheless, in this scenario it would also be expected that there would be more parasitism in the outermost cells (since the parasitoid would presumably have had more time to locate the active site), although the data from the regression analysis indicate the opposite trend. Several studies have proven that the daily and seasonal asynchrony between the activity of the parasitoid and host may significantly modify the probability of nest attack by the parasitoid (Rosenheim, 1989; Strohm *et al.*, 2001). Similarly, the degree of aggregation of the host nests could increase or reduce the rate of parasitoidism as a function of whether the relationship is directly density-dependent or inversely density-dependent (Rosenheim, 1990; Strohm *et al.*, 2001). Since there are no data concerning the start of provisioning of the nests, nor the greater or lesser proximity (aggregation) of the nests attacked, these hypotheses cannot be tested. In any case, some studies have suggested that the activity of parasitoids could be more affected by decreases in temperature, because such parasitoids are usually smaller than their host and more dependent on high ambient temperatures (Willmer, 1985; Rosenheim, 1990). If, as would be logical, the nests with a

greater number of cells had been started early in the nesting season, the environmental conditions could have been more restrictive for the parasitoids at the end of the season, resulting in less parasitism of the last provisioned cells (the outermost ones).

The analysis also reflects an influence of the number of cells on the mortality rate, such that this rate is higher for a smaller number of cells, later tending to become reduced in nests with more cells. However, it should be borne in mind that in the present work we only used nests from which at least one adult had been obtained, with which all nests with a single cell showed a zero mortality rate. In light of the above, it is likely that the increase in the number of cells would lead to a reduction in the mortality rate. In view of the behaviour of M1 and MP towards the inside of the nests, the former being reduced and the latter increased (M2 did not show a definite trend), and considering that the influence of M1 (23.5% mortality) was greater than that of MP (12.6% mortality) for the population studied, a reduction in overall mortality would be expected as the number of cells increases. Nevertheless, it should be taken into account that the situation might not be the same for other populations that must face up to different environmental and biological factors.

### Vestibular Cell

The fact that almost 75% of the nests had a VC, together with the observation that the VC is associated with IFOM and AM nests, suggests that VC is probably a completion structure, which the females build on finishing a nest. Accordingly, the absence of VC could reveal unfinished nests, which for some reason had not been completed. Despite this, the role of this structure remains essentially unknown. It has been proposed that VC might have arisen as an adaptation to reduce the access of natural enemies into the nest (Krombein, 1967; Coville and Coville, 1980; Coville, 1982; Coville and Griswold, 1984), although Krombein (1967) considered that—nowadays—VCs are behavioural relicts, since there are no natural enemies to oviposit through the mud walls. In a study carried out with *Osmia rufa*, Seidelmann (1999) failed to find a relationship between the presence or length of VC and a reduced degree of parasitism; other studies have also been unable to find any relationship between these variables (García and Adis, 1995; Weaving, 1995). The results reported here show that the presence of a VC does not lead to a reduction in the rate of parasitoidism; indeed, all the nests attacked had this structure. However, we did see a reduction in the M1 mortality rate in the nests with a VC, which suggests the role of these structures as a buffer. This latter possibility had been proposed by Seidelmann (1999),

although no data to support such a hypothesis were provided. Accordingly, the VC could be a structure established at the end of the nest, whose presence would lead to a reduction in mortality not associated with parasitoids.

The length of the VC varied considerably and analysis of this revealed the significant influence of the separation of the last provisioned cell and, although to a lesser extent, of the available space. The mean size of the VC was clearly greater than that of the provisioned cells, such that the possibility that one might be dealing with a space outside the nest, insufficient to harbour a cell, should be discarded. In view of the presumably strong competition that exists in natural environments among species unable to excavate plant material, to locate suitable cavities for nesting purposes (Danks, 1971; Fye, 1972; Tepedino *et al.*, 1979; Sears *et al.*, 2001), the failure to use an space large enough for harbouring a cell seems paradoxical. One possible explanation for this could be that there might be an optimum number of cells for each type of nest, such that -regardless of the space available- once this optimum has been reached and the VC has been established, the length of the VC would be matched to the remaining space to limit the possibility of supersedure. In a scenario of strong competition for the location of cavities, supersedure may be common. Such supersedure, which involves the establishment of nests (by individuals of the same or another species) on the outside of other nests that have already been completed, could lead to an increase in the mortality of the individuals of the innermost cells, if they were unable to go through the partitions of the external nest during their emergence. The relationship observed between the remaining space outside the last provisioned cell and the length of the VC (the greater the remaining space, the longer the VC; see coefficients in Table IV) suggests a role in the limitation of this space.

If the VC was considered to be a completion structure, its presence in AF nests, presumably incomplete, would be surprising. The GLM analysis did not reveal a statistically significant influence of the type of nest on the length of the VC. However, it did show that the VC of the AF nests was shorter than that of the IFOM or AM nests; additionally, the length of these VCs was similar to that of the provisioned cells in AF nests, while VCs being much longer than provisioned cells in AM and IFOM nests. This means that the VCs of AF nests could simply be cells that had not been provisioned for unknown reasons, and not completion structures.

### **Empty Intercalary Cells**

EICs appear intercalated among provisioned cells in different species of rubicolous hymenoptera (Krombein, 1967; Coville, 1982; Jayasingh and

Taffe, 1982; Sears *et al.*, 2001). Their presence is generally occasional, less than that of VCs, and it has been suggested that they could arise following detection by the females of parasitized or fungal spore-containing cells, or that they could correspond to a VC, reflecting the start of a new nest by a superseding wasp. They could also be behavioural relicts of ancestors that established nests constituted by only one provisioned cell and one vestibular cell when adapted to a more optimal occupation of longer cavities (Krombein, 1967).

Comparison of the sizes of VCs and EICs revealed, however, that the EICs were significantly smaller than the VCs, although they did not differ in size from the provisioned cells. This contradicts the hypothesis that the EICs could correspond to VCs. Nevertheless, in some species of the genus *Trypoxylon*, EICs with sizes similar to or even larger than VCs have been reported (Assis and Camillo, 1997), such that it cannot be discarded that there may be different “types” of EICs, with different origins and functions. Also, no relationship was found between the presence of EICs and the existence of MP or M1 cells in the nest, so neither does it seem to be very clear that EICs would have derived from the detection of “anomalous” cells in the nest by the female.

The logistic regression analysis revealed only the available space and the number of occupied cells as influential variables: the longer cavities had a greater probability of having EICs, while as the number of occupied cells increased, the presence of EICs decreased. Additionally, an “aggregated” distribution of the EICs was found, such that some nests tended to have several EICs whereas in many other cases (more than would be expected by chance) this type of structure did not appear. This suggests that EICs may be established by certain females exhibiting “abnormal” behaviour, leaving some cells unprovisioned. In this situation, it would also be logical to surmise that the total number of cells in nests with EICs would be similar to that of nests without EICs (whereas the number of occupied cells would, logically, be greater in the latter), which is precisely what was observed in the present study. The causes of such abnormal behaviour are unknown, although it seems clear that there is no relationship between the presence of EICs and the existence, in the same nest, of cells with MP or M1.

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