

**Research article**

**Intraspecific variation in the comb structure of  
*Polistes dominulus*: parameters, maturation, nest size  
and cell arrangement**

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**Key words:** *Polistes dominulus*, nest architecture, intraspecific variation, building behaviour.

**Summary**

The intraspecific variability of the nest structures of *Polistes dominulus* is described quantitatively. Several variables already used in the literature (e.g. wall expenditure and diameter ratio) are reexamined and a number of new ones are suggested. Variables are analyzed with respect to their dependency on the size and the maturation of the colony.

The analyses were based on mathematical functions deduced for ideal nest growth. In the examined species, the numbers of outer walls and buildable cells do not differ significantly from those calculated for the ideal growth, demonstrating that the real nests are close to the “ideal” round form.

*P. dominulus* builds compact and well-centered nests. The shape of the comb remains compact during the maturation of the nest. The eccentricity changes during maturation: it is significantly greater in the mature stage than in younger combs.

Analysis reveals that the wasps prefer to initiate a new cell in a position where it will have at least three neighbors. The nests in different age groups not only differed in height, but were also under the control of three different constraints: the structure itself, the large larvae and indirectly the meconium.

**Introduction**

Most eusocial wasps build combs from vegetable fiber and oral secretion (Wenzel, 1991). The comb consists of a particular unit, the cell, which is generally hexagonal and contains only one offspring. This modular and regular structure is characteristic among social wasps and some bees, but lacking for the other social insects such as ants and termites. Despite this modular construction, the combs of wasps display great diversity, both inter- and intraspecifically.

The literature on social wasps abounds in nest descriptions, most of which are based on small numbers of nests. Nevertheless, the available qualitative data were sufficient for a generic level phylogeny of the social wasps to be constructed purely

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on the basis of nest structures (Wenzel, 1991; 1993). Even so, the intraspecific or intrageneric variation remains largely unknown due in part to the lack of suitable parameters (Downing and Jeanne, 1986). The verbal descriptions and/or the illustrations presented by certain authors generally provide a nice impression of the nests (e.g. Yamane and Makino, 1977; Kojima, 1982), but they can hardly be used for quantitative comparison or further analysis.

Surprisingly little effort has been made to quantify nest parameters. The simplest and most common method is to give the dimensions of the comb and count ratios or products from these parameters (e.g. Yamane and Makino, 1977; Kojima and Jeanne, 1986; Kojima, 1988). Yamane (1984) formulated the comb as **mL-P-nR**, where **P** is the vertical row containing the petiole and **m** and **n** are the numbers of rows to the left or right of **P**, respectively. For their comparative analysis, Downing and Jeanne (1986) measured several parameters (mainly angles and distances) on 12 *Polistes* species. Using circular statistics Wenzel (1989), was able to test the eccentricity of nests. The greatest effort toward quantification was made by Ishay et al. (1982), who used the hexagonal cell and the round comb as the basis of quantification. Unfortunately, their analysis contains errors (which were partly corrected by Wenzel (1989)), and they analyzed only the "wall expenditure", i.e. the wall/cell ratio.

The purpose of the present paper is to describe the intraspecific variability of the nest structure of the paperwasp *Polistes dominulus*. Quantitative variables are used for the analyses. We both re-examine the commonly used variables (e.g. wall expenditure and eccentricity) and define new ones. We address the following questions. 1. How do nest size and maturation affect the nest structure? 2. How closely do the cell arrangements of actual nests match the ideal (analytically derived) structure? 3. How is the building behavior reflected in the structure?

## Materials and methods

168 nests of *P. dominulus* wasps were reared in the laboratory (Ethological Laboratory of C.N.R.S. (UPR 38) in Marseille, France) under the same conditions. The rearing cages were plastic boxes (25 · 15 · 15 cm), containing food (*Noctuidae* caterpillars and candy sugar), water and building paper. The average temperature in the rearing room was around 25 °C, but every cage was illuminated and warmed further to 35 °C with an incandescent lamp (40 watt) from 8 a.m. to 8 p.m. The substrates of the nest were removable plastic disks, which were set horizontally in the middle of the cages. In this study, only dry nests were analyzed. Every data point in our analysis reflects an independent structure. This means that every nest was built by different wasps, with no chronological connection.

The ages (maturation phases) of the structures were assessed by utilizing the landmarks of different developmental stages of the brood in the nest. Four qualitative categories were distinguished:

1. *Embryo* nest. The nest is small and its age is only a few weeks. This stage lasts from nest initiation to the moment when the largest larva defecates into the cell.
2. *Larval* nest. At least one cell contains meconium, but not residual pupal silk. The development of the oldest pupae, if any, is not yet finished (pupal caps are closed).

3. *Premature* nest. Opened pupal caps or pupal silk residue can be found in at least one of the cells.
4. *Mature* nest. After the emergence of the first 6 workers, we considered the nest to be *Mature* (subsequent workers have few contacts with higher ranked individuals and the brood; they are the reserve work-force of the colony (Theraulaz et al., 1992)). The number of opened pupal caps, silk rings (pupal cap residuals) and the layers of the meconium in the cells distinguish these nests from those in the previous group.

## Results

### *Structure of the comb: idealized growth and basic relationships*

The unit of the nest is the cell. Cells are arranged in rows, forming a slender or discoid comb. The combs of *P. dominulus* are characteristically round. The development of these combs proceeds similarly to ideal growth from a center by the addition of circles of cells to the previous structure. Setting out from these qualitative findings, the analysis begins from the idealized growth of ideal (regular) combs.

Consider a regular comb structure composed of rings ( $r=0, 1, \dots, R$ ) of regular hexagonal cells, with an initial cellular core ( $r=0$ ). The building proceeds through the successive addition of rings to the initial core. The size of the comb therefore can be expressed as the number of rings. In consequence of this type of growth, the ideal comb structure remains compact and regular (six-order center of symmetry). In this structure, the numbers of total cells ( $N$ ) and walls ( $Z$ ) can be computed as a function of the ring number ( $R$ ) (see Appendix I):

$$N(R) = 1 + 3 \cdot R \cdot (R + 1) \quad (1)$$

$$Z(R) = 6 + 3 \cdot R \cdot (3 \cdot R + 5) \quad (2)$$

Every new ring is built on the circumference of the comb, which consists of  $S$  walls:

$$S(R) = 6 \cdot (2 \cdot R + 1) \quad (3)$$

Onto the outer walls ( $S$ ), a given number of new cells ( $B$ ) can be built, and these constitute the new ring ( $R + 1$ ):

$$B(R) = 6 \cdot (R + 1) \quad (4)$$

Since every parameter is a function of  $R$ , we can interpret  $S$  and  $B$  as functions of  $N$ . In this case, the size of the ideal comb can also be expressed in terms of  $N$  (Appendix I; Péntzes and Karsai, 1993):

$$S(N) = 2 \cdot \sqrt{3} \cdot \sqrt{4 \cdot N - 1} \quad (5)$$

$$B(N) = 3 + \sqrt{3} \cdot \sqrt{4 \cdot N - 1} \quad (6)$$

Although these expressions are accurate only for ideal combs with closed rings, the functions permit an appropriate interpolation between two values given by expressions (3) and (4) for all values of  $N$ , and they are easily tractable too.

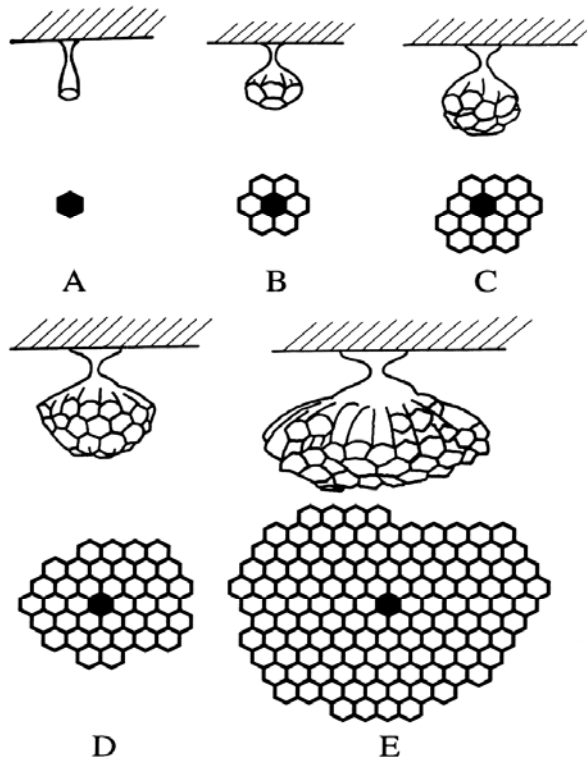
The wall/cell ratio or the average wall number per cell ( $W$ ), can be deduced from expressions (1) and (2):  $W = Z/N$  (7)

and

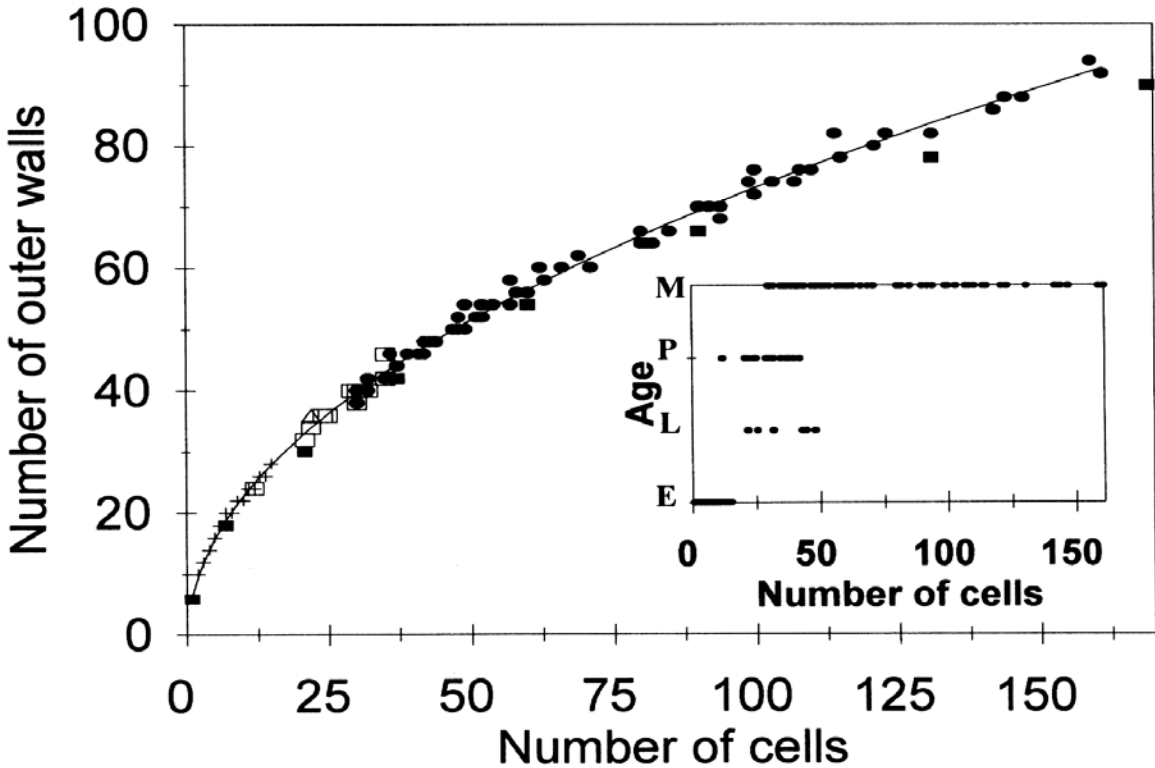
$$\lim_{R \rightarrow \infty} W = \frac{6 + 3 \cdot R \cdot (3 \cdot R + 5)}{1 + 3 \cdot R \cdot (R + 1)} = 3.0$$

### *The nest structure of P. dominulus*

The nest of the wasps are three-dimensional structures. The comb can be regarded as arranged cell rows, where the walls have different heights. In general, the modifications in cell arrangement (cell initiation) and wall height (lengthening) are linked to the different building decisions by different individuals (see for example West Eberhard, 1969; Pratte, 1990). Purely by studying the cell arrangement in a two-dimensional framework, we can quantitatively express the design (compact, elongated or round) of the nests. For this approach, we assume that every initiated cell is of the same size. Similar simple hexagonal nest maps are commonly used by students of wasps to facilitate data registration and evaluation (Fig. 1).



**Figure 1.** Side view and nest map of the nest of *P. dominulus*. A) unicellular *Embryo* nest; B) multicellular *Embryo* nest; C) *Larval* nest; D) *Premature* nest; E) *Mature* nest



**Figure 2.** Number of outer walls as a function of cell number in *P. dominulus*. Every point represents an independent structure. Symbols and acronyms: E and +: *Embryo* nests; L and Δ: *Larval* nests; P and □: *Premature* nests; M and ●: *Mature* nest; ■: *Ideal* nest. Detailed description of the categories in the text. Solid line: fitted curve according to the power function in expression (8) (see also Table 1). Inset: Scattergram of nest size (cell number) and age (categories)

#### *Relationship between nest size and age*

As may be expected, nests belonging to different age categories differ in size (cell number) (Kruskal-Wallis test,  $p < 0.001$ ,  $n = 168$ ). The nest of every age group differed significantly (Mann-Whitney test,  $p < 0.005$ ) in size, except for the *Larval* and the *Premature* nests ( $p > 0.2$ ). The *Embryo* nests had small combs (median = 6.0,  $n = 76$ ), which consisted of 15 or fewer cells (Fig. 2). There were no combs consisting of between 15 and 21 cells, and thus we can conclude that the *Embryo* stage was generally accomplished before the nest reached 20 cells. In one nest, the comb remained small (12 cells), but workers emerged from it. This may be a special case, when the egg-laying by the foundress was low or ceased, although the brood care was continued. Between the *Larval* (median = 37.5,  $n = 6$ ) and the *Premature* phases (median = 31.0,  $n = 21$ ) the nests did not increase significantly in size and the combs did not exceed 48 cells in number. The *Mature* nests exhibited a great variation in size ranging from 30 to 161 cells (median = 61,  $n = 64$ ).

### Measurements to describe the cell arrangement of two-dimensional nest structures

#### Outer walls

The circumference of the comb is composed by the outer walls of the peripheral cells. The number of outer walls of an ideal nest can be calculated via expression (5). In a natural nest, these can easily be counted. Elongated combs and combs with lobes have larger  $S$  values than compact and round combs with the same cell number.

The combs of *P. dominulus* have small circumferences (Fig. 2). The number of outer walls of real nests seemed to be slightly higher than the minimum values, calculated for ideal combs. On the basis of function (5), we hypothesized that  $S$  for natural nests depends on  $N$ , as follows:

$$S(N) = a \cdot (4 \cdot N - 1)^b \quad (8)$$

The  $S$  values of the nests fit the values predicted from this function very well (Table 1). The high value of the coefficient of determination ( $r^2$ ) indicated that the number of outer walls is highly dependent on the number of cells. The regression coefficient ( $b$ ) of this fitting did not differ significantly (t test,  $p > 0.1$ ) from that calculated for idealized growth. The values of  $S$  for different age groups differed significantly (Mann-Whitney test,  $p < 0.005$ ). The only exception was observed for the *Larval* and the *Premature* combs (Mann-Whitney test,  $p > 0.2$ ), which did not differ in cell number.

The wall/cell ratio ( $W$ ) behaved similarly to  $S$ . There was no significant difference in  $W$  between the *Larval* and the *Premature* combs (Mann-Whitney test,  $p > 0.2$ ). All natural nests had ratios very close to the minimum value of  $W$  calculated via expression (7). The ratio decreased with cell number as predicted by (7), and approximated the theoretical minimum value (3.29 for the largest nest, with  $N = 161$ ).

#### Compactness

The number of walls for a given comb size depicts the shape of the comb well, but it can not indicate the difference between two combs in which the position of a cell is the only difference. In this case, the length of circumferences of the combs are the same, but the two structures are not identical in cell arrangement. This difference can be expressed by a parameter called compactness ( $C$ ) (Fig. 3).

The first step in calculating  $C$  is to determine the geometric center of the comb. In the nest map, where  $N$  is the number of cells (regular identical hexagons) and  $(x_i, y_i)$  are the coordinates of the center of cell  $i$  ( $i = 1, \dots, N$ ) the coordinates of the geometric center are:

$$(x_e = 1/N \cdot \sum_i \cdot x_i, y_e = 1/N \cdot \sum_i \cdot y_i).$$

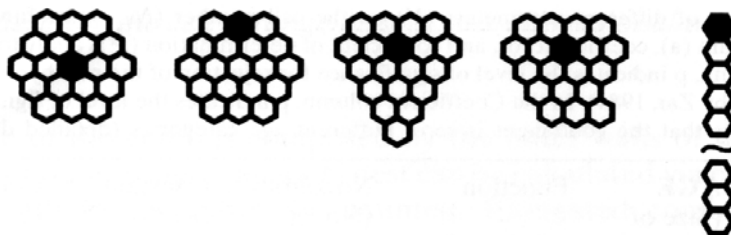
The distances of all cells from this center are then summed to obtain the  $C$  value:

$$C = \sum_i \sqrt{(x_i - x_e)^2 + (y_i - y_e)^2} \quad (9)$$

**Table 1.** Regression of different parameters ( $Y$ ) on the cell number ( $N$ ). If the function is not linear, the parameters (constant ( $a$ ), coefficient ( $b$ ), and coefficient of determination ( $r^2$ )) apply to the linearized form. In the Function column,  $p$  indicates the level of significance for rejection of the null hypothesis that the relationship is linear (test by Zar, 1984). In the Coefficient column,  $p$  indicates the level of significance for rejection of the null hypothesis that the coefficient is zero. Different age categories (detailed description in the text):

Parameter ( $Y$ )	AGE (Size of nests)	Function	No. of obs. (No. of nests)	Constant ( $a$ )	Coefficient ( $b$ )	Coeff. Deter. $r^2$
Outer walls	<b>E, L, P, M</b> (6-161)	$Y = a \cdot (4 \cdot N - 1)^b$ $p > 0.05$	131	1.27	0.51	0.97
Compactness	<b>E, L, P, M</b> (6-161)	$Y = a \cdot N^b$ $p > 0.1$	131	-1.07	1.51	1
Buildable cells	<b>E, L, P, M</b> (6-161)	$Y = a \cdot (4 \cdot N - 1)^b$ $p > 0.05$	131	0.08	0.54	0.99
NS3	<b>L, P, M</b> (21-161)	$Y = b \cdot N + a$ $p > 0.1$	91	1.49	0.045 $p < 0.005$	0.47
NS3	<b>L, P</b> (21-48)	$Y = b \cdot N + a$ $p > 0.1$	27	-0.17	0.009 $p > 0.05$	0.13
NS3	<b>M</b> (30-161)	$Y = b \cdot N + a$ $p > 0.1$	64	1.8	0.042 $p < 0.005$	0.48
NS2	<b>E, L, P, M</b> (6-161)	$Y = a \cdot (4 \cdot N - 1)^b$ $p > 0.1$	131	0.32	0.45	0.82
NS2	<b>L, P, M</b> (21-161)	$Y = b \cdot N + a$ $p > 0.1$	91	8.23	0.126 $p < 0.005$	0.72
Diameter ratio	<b>E, L, P, M</b> (6-161)	$Y = b \cdot N + a$ $p > 0.1$	130	0.85	0.000 $p > 0.1$	0.05
Eccentricity	<b>E, L, P</b> (1-48)	$Y = b \cdot N + a$ $p > 0.1$	67	0.47	0.025 $p > 0.1$	0.02
Eccentricity	<b>M</b> (30-161)	$Y = b \cdot N + a$ $p > 0.1$	64	0.54	0.031 $p > 0.01$	0.08
Height	<b>E, L, P, M</b> (1-161)	$Y = a \cdot \ln(b \cdot N)$ $p < 0.001$	166	6.95	1.7	0.86
Height	<b>E</b> (1-15)	$Y = b \cdot N + a$ $p > 0.005$	75	9.99	0.386 $p < 0.005$	0.61
Height	<b>P</b> (12-42)	$Y = b \cdot N + a$ $p > 0.1$	21	26.16	0.094 $p > 0.1$	0.06
Height	<b>M</b> (30-161)	$Y = b \cdot N + a$ $p > 0.05$	64	27.92	0.047 $p < 0.005$	0.33

**E:** Embryo; **L:** Larval; **P:** Premature; **M:** Mature



Variables					
$N$	19	19	19	19	19
$S$	30	30	32	32	78
$W$	3.79	3.79	3.84	3.84	5.05
$B_1$	6	6	6	7	6
$B_2$	12	12	13	11	36
$B_3$	0	0	0	1	0
$E$	0	1.73	0.73	0.69	9
$C$	28.39	28.39	29.67	29.30	90

**Figure 3.** The values of different variables for different-shaped 19-cell nests. The black cell denotes the first cell.  $N$  = number of cells;  $S$  = number of outer walls;  $W$  = wall/cell ratio;  $B_1$ ,  $B_2$ ,  $B_3$  = potentially buildable cells with one, two and three neighbors, respectively;  $E$  = eccentricity;  $C$  = compactness. For a detailed description of the variables, see the text and Appendix I

measured in units determined by the distance between the centers of two adjacent cells. For nests with the same cell number, round combs have the lowest  $C$  value, and these structures are therefore the most compact.

The compactness data on real nests of *Polistes* were fitted to a simple power function of the cell number:

$$C(N) = a \cdot N^b \quad (10)$$

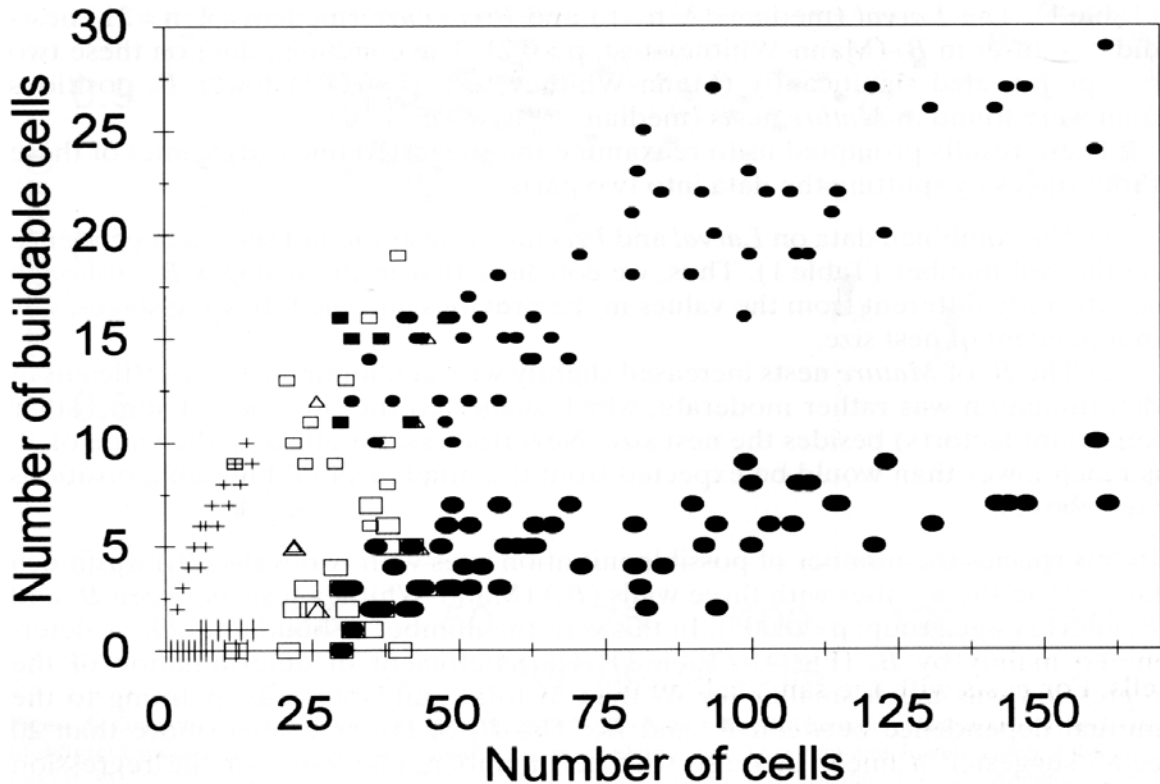
The high value of the coefficient of determination ( $r^2$ ) indicated that the  $C$  was highly dependent on the number of cells (Table 1). Although the real data were very close to the theoretical minimum values for regular round combs, the regression coefficient ( $b$ ) of this fitting differed significantly (t test,  $0.01 > p > 0.001$ ) from that calculated for idealized growth.

Similarly to the  $S$  variable, the different age groups differed significantly from each other in  $C$  (Mann-Whitney test, for all pairs,  $p < 0.005$ ), except in the case of the *Larval* and the *Premature* combs ( $p > 0.2$ ), which did not differ in cell number. In both characteristics, the actual values of the variables for a given nest were determined by its size (the values increase with cell number) rather than its age (see, for example, the values for a small (12 cells) *Premature* comb in Fig. 2).

### Potentially buildable cells

Since the comb can increase in cell number only on its circumference, this part of the comb is particularly important. With consideration of building constraints, we can calculate the number of buildable new cells (potential cell initiation positions) ( $B$ ) of a nest of given size and form (Fig. 3). This variable can be important if we wish to gather information about the building script of the given species. To observe the number of potential cells with different ready wall numbers (we define a ready wall as a wall on the periphery of the nest which can be used to complete a





**Figure 4.** Number of buildable cells as a function of cell number in *P. dominulus*. Symbols as in Fig. 2. Small symbols: buildable cell with two walls ( $B_2$ ); large symbols: buildable cells with three walls ( $B_3$ )

new cell), we can estimate statistically, for example, the chance of the site of the next initiation.

The  $B$  values of the nests of *P. dominulus* fit a similar power function to that described by expression (8) very well (Table 1). The high value of the determination coefficient indicated that the number of buildable cells is highly dependent on the number of cells. The regression coefficient ( $b$ ) of this fitting did not differ significantly ( $t$  test,  $p > 0.2$ ) from that calculated for idealized growth.

Our own observations and literature findings (Karsai and Theraulaz, 1995; and observations on building by *P. fuscatus*: Downing and Jeanne, 1988; 1990) led us to assume that the wasps build new cells where at least two ready walls are present to complete a new cell (the initiation of first and second cells was therefore also neglected). In our nests we did not find buildable cell positions with four or more ready walls, and we therefore omitted these possibilities from the analyses. Hence, for *P. dominulus* we considered only buildable cells possessing two ( $B_2$ ) or three ( $B_3$ ) ready walls. The small *Embryo* combs contained no or only one three-walled initiation positions (median = 0,  $n = 76$ ). This group has significantly smaller  $B_3$  values than those of the others (Mann-Whitney test,  $p < 0.005$ ). After the *Embryo* stage, the number of potential initiation sites with three walls increased (Fig. 4). The three older stages together showed a slight linear increase in  $B_3$  with the cell number

(Table 1). The *Larval* (median = 3,  $n = 6$ ) and *Premature* (median = 2,  $n = 21$ ) nests did not differ in  $B_3$  (Mann-Whitney test,  $p > 0.2$ ). The combined data on these two groups revealed significantly (Mann-Whitney test,  $p < 0.005$ ) fewer  $B_3$  positions than were found in *Mature* nests (median = 5,  $n = 64$ ).

These results prompted us to reexamine the suggested linear dynamics of these three stages by splitting the data into two parts:

1) The combined data on *Larval* and *Premature* nests did not show a dependence on the cell number (Table 1). Thus, we conclude that in these stages  $B_3$ , although significantly different from the values in the previous and the following stages, was independent of nest size.

2) The  $B_3$  of *Mature* nests increased slightly with cell number. The coefficient of determination was rather moderate, which suggested the presence of some other important factor(s) besides the nest size. Nevertheless, for all sizes, the value of  $B_3$  is much lower than would be expected from the number of all buildable positions (see above).

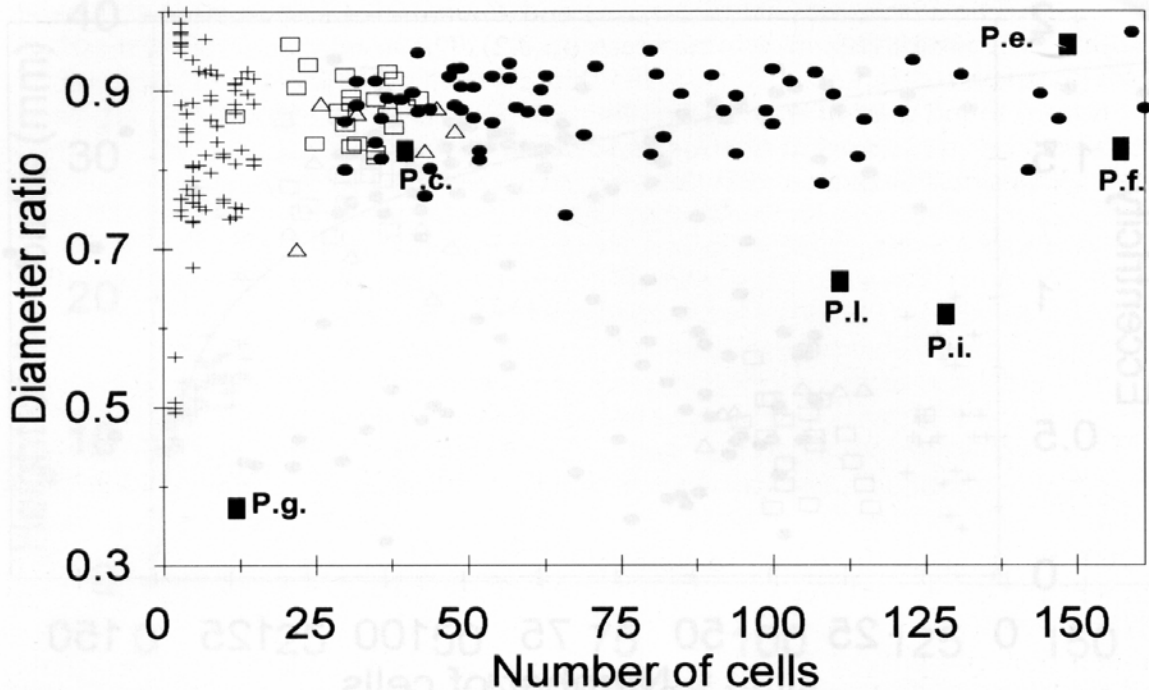
In this species the number of possible initiation sites with two walls ( $B_2$ ) was much larger than the number with three walls ( $B_3$ ) (Mann-Whitney tests between  $B_2$  and  $B_3$  in every age group:  $p < 0.005$ ). In this way, the number of buildable cells is determined mainly by  $B_2$  (Fig. 4) (Table 1). The coefficient of determination of the regression was much smaller for  $B_2$  than in total buildable cells, pointing to the mutual dependence between  $B_2$  and  $B_3$ . The  $B_2$  of larger combs (more than 20 cells) suggested a linear increase with cell number. The value of the regression coefficient for  $B_2$  was significantly larger (t test,  $p < 0.001$ ,  $n = 182$ ) than for  $B_3$  (Table 1).

### Diameter ratio

The shape of the comb can be evaluated more simply in terms of the ratio of the longest and shortest dimensions of the comb. In the case of an elongated nest, it is easy to distinguish the longest axis (and give the width/length ratio), but it is sometimes difficult in the case of round combs. To attain a value of the diameter ratio between zero and one, which refers to the "elongatedness" of the comb, it is reasonable to use the longest diameter or axis as denominator. The numerator should be the longest width perpendicular to the longest axis.

For *P. dominulus* with the exception of some small combs the diameter ratios were generally between 0.7 and 1.0 (Fig. 5). The diameter ratio was independent of the nest size (Table 1). The values of the ratio did not differ significantly from age group to age group (Kruskal Wallis test,  $p > 0.05$ ). Even the *Embryo* combs, where one new cell may strongly affect the ratio because of the disproportionate increase in one direction, were rather round (median = 0.83,  $n = 76$ ).

Recalculation of the nest dimensions reported by Downing and Jeanne (1986) allowed comparisons of the *Mature* nest shapes of different *Polistes* species as well (Fig. 5). *P. canadensis* and *P. exclamans* exhibited diameter ratios similar to that of *P. dominulus* (median = 0.88,  $n = 64$ ). The other species presented in the graph generally built more elongated structures.



**Figure 5.** Diameter ratio as a function of cell number. Symbols as in Fig. 2. The symbol ■ denotes the positions of different species from *P. dominulus*. Data were recalculated from Downing and Jeanne (1986, Table IV). Letters in Figures denote the following species: P.f.: *P. (Fuscopolistes) fuscatus*, P.e.: *P. (Aphanilopterus) exclamans*; P.l.: *P. lanio*; P.i.: *P. instabilis*; P.g.: *P. goeldii*; P.c.: *P. canadensis*

### Eccentricity

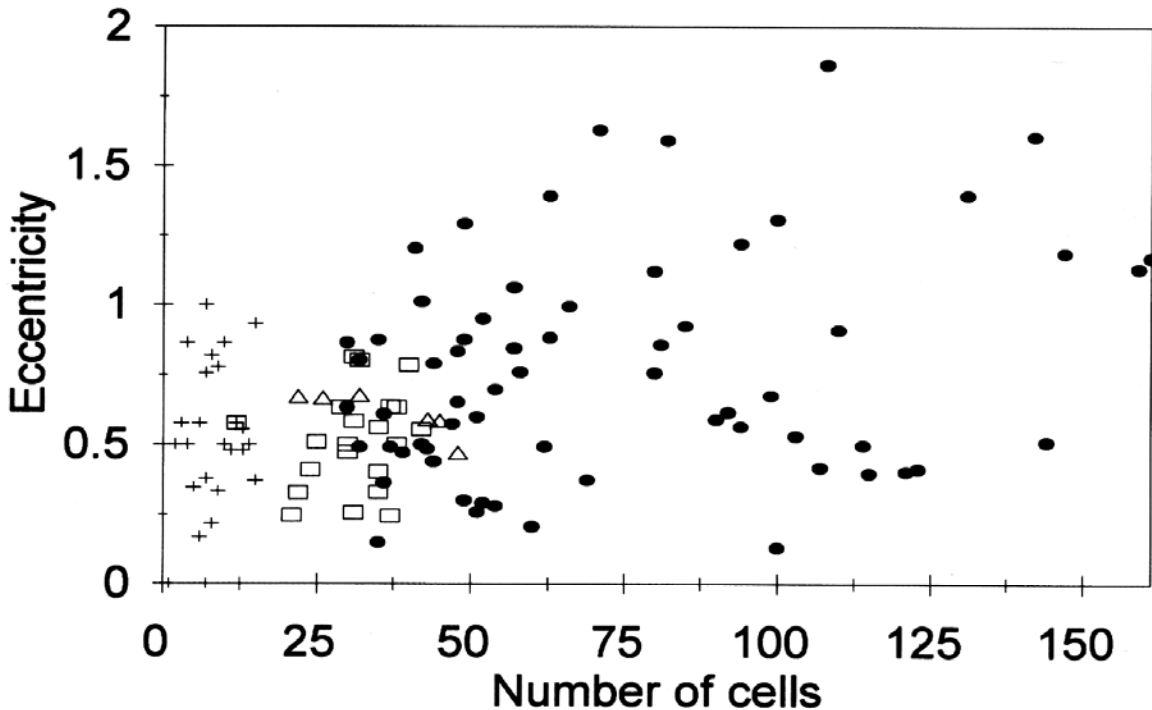
The combs hang from one or more petioles from the substrate or another comb. If two or more petioles are built, the first or primary petiole is used as the main support of the comb and a series of cells are built around it before secondary petioles are constructed. The comb can be centered or off-centered from this petiole (below we discuss only the primary petiole). The localization of the petiole and comb attachment (first cell) for a simple comb is generally easy.

Eccentricity ( $E$ ) reflects the uniformity of the distribution of the cells around the petiole. The eccentricity of a comb is the distance (measured in the same units as compactness (see above)) between the petiole attachment (first cell) and the geometric center of the comb:

$$E = \sqrt{(x_e - x_0)^2 + (y_e - y_0)^2} \quad (11)$$

where  $(x_e, y_e)$  and  $(x_0, y_0)$  are the coordinates of the geometric center (see above) and the center of the first cell, respectively.

The nests of *P. dominulus* were well centered (Fig. 6). The actual values and variations were far from the theoretically possible upper limit. The nests relating to different age categories differed in eccentricity (Kruskal Wallis test,  $p < 0.001$ ,



**Figure 6.** Eccentricity as a function of cell number in *P. dominulus*. Symbols as in Fig. 2

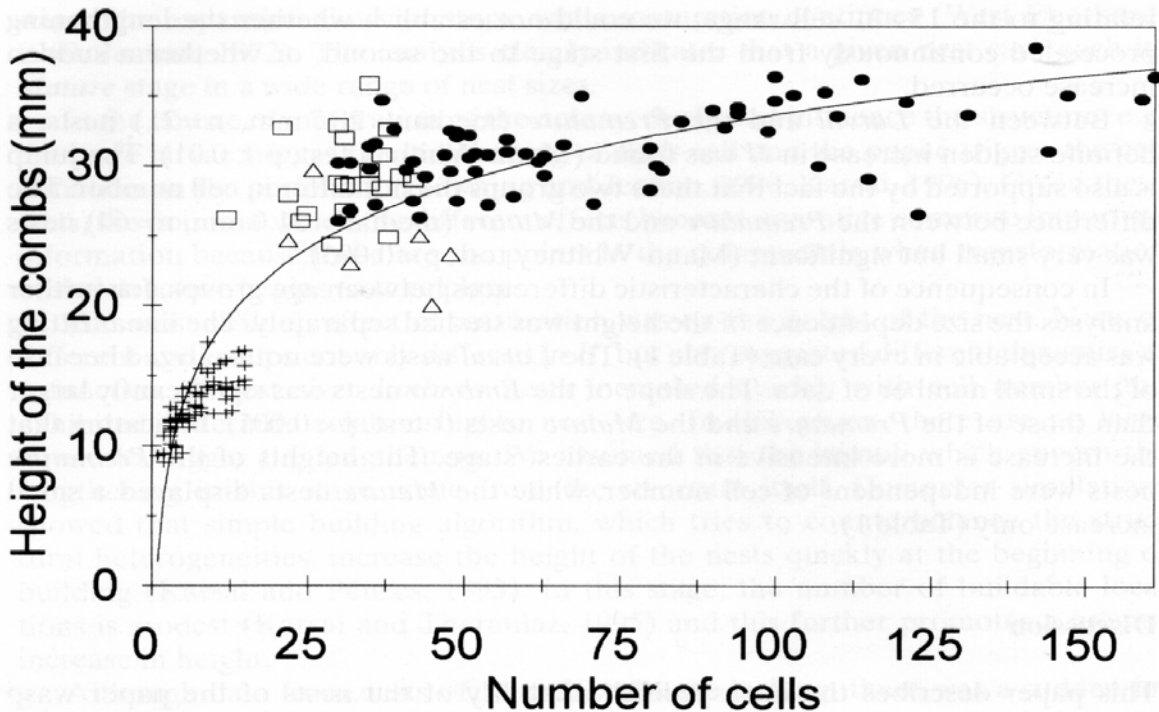
$n = 167$ ). The data seemingly formed two groups: the first group represents *Embryo*, *Larval* and *Premature* nests (median = 0.50,  $n = 103$ ), and the second *Mature* nests (median = 0.69,  $n = 64$ ). The  $E$  of these two groups differed significantly (Mann-Whitney test,  $p < 0.001$ ,  $n = 167$ ). Linear fitting of  $E$  to cell number revealed that in both groups  $E$  was independent of nest size. The regression constants showed (as did the medians above) that *Mature* combs were slightly more eccentric than younger ones (Table 1). The variability in  $E$  values was larger in the *Mature* combs too (Fig. 6).

When the test proposed by Wenzel (1989) was applied only 9 of 80 tests differed significantly ( $p < 0.05$ ) from the radially symmetrical cell arrangement ( $\chi^2$  test for 6 ( $60^\circ$ ) radial sectors from the petiole,  $n = 80$  nests of more than 30 cells). All of these nests were *Mature* and displayed the largest  $E$  values. In the examined size range, we found no significant correlation ( $r = 0.073$ ,  $p > 0.1$ ,  $n = 166$ ) between the eccentricity and diameter ratio.

Although the aim of this study was merely to describe the intraspecific variability in the round nests of one species in a two-dimensional framework, the evaluated variables can be used in all simple wasp nest forms. We present a series of examples to demonstrate the sensitivity and scale of the main parameters for identically sized nests with different forms (Fig. 3).

#### *The third dimension of the nest: the height*

The height of the nest (from the substrate to the top of the highest cell) can yield important information if we study the lengthening behavior of the wasps. The



**Figure 7.** Height of the comb as a function of cell number in *P. dominulus*. Symbols as in Fig. 2. Solid line: fitted curve according to the expression (12) (see also Table 1)

height of the comb is generally determined by the largest (longest) cell and the curvature of the base of the comb. Measurement of this parameter with a slide-gauge is generally easy and unambiguous.

In *P. dominulus*, the height of the comb did not increase independently of the nest size: the height increase seems to diminish with cell number (Fig. 7). It is assumed that the lengthening is independent of the stimuli originating from the structure, i.e. as the nest grows in size, the chance of the largest cell lengthening decreases. Accordingly we hypothesized that the increase in height ( $H$ ) is inversely proportional to the cell number ( $N$ ):

$$H = a \cdot \ln(b \cdot N) \quad (12)$$

The model was rejected (see Table 1, Function column) even though the coefficient of determination was high. We concluded that the increase in height of the nests did not depend on the nest size in this manner.

The wasps may use external references for the lengthening (the stimuli do not stem from the cell structure itself, but from another source, e.g. from the brood or the mental blueprint). Different dynamics may be involved in different maturation phases, which contradicts the "global" approach presented above. The different age groups had different  $H$  (Kruskal Wallis test,  $p < 0.001$ ). The  $H$  of the *Embryo* nests (median = 12.5 mm,  $n = 75$ ) were significantly less than those of the *Larval* (median = 24.2 mm,  $n = 6$ ) nests (Mann-Whitney test,  $p < 0.001$ ). Because of a lack of data

relating to the 15–21 cell range, we could not establish whether the lengthening proceeded continuously from the first stage to the second, or whether a sudden increase occurred.

Between the *Larval* and the *Premature* (median = 28.7 mm,  $n = 21$ ) nests, a definite sudden increase in  $H$  was found (Mann-Whitney test,  $p < 0.01$ ). The jump is also supported by the fact that these two groups did not differ in cell number. The difference between the *Premature* and the *Mature* (median = 31.0 mm,  $n = 64$ ) nests was very small but significant (Mann-Whitney test,  $p < 0.005$ ).

In consequence of the characteristic differences between age groups, for further analyses the size dependence of the height was studied separately. The linear fitting was acceptable in every case (Table 1). The *Larval* nests were not analyzed because of the small number of data. The slope of the *Embryo* nests was significantly larger than those of the *Premature* and the *Mature* nests ( $t$  test,  $p < 0.001$ ), indicating that the increase is more intensive in the earliest stage. The heights of the *Premature* nests were independent of cell number, while the *Mature* nests displayed a small increase only (Table 1).

## Discussion

This paper describes the intraspecific variability of the nests of the paper wasp *P. dominulus*, covering the natural range of the nest size (1–161) of this species. Our aim was not to describe the natural variability, which is due to uncontrolled factors (e.g. the influence of different substrates or spatial obstacles), but to study the “inherent variance” and to give the basic relationships between these and the size and the age of the nests. The two-dimensional representation of the nest structure, which reveals only the positions of cells and the petiole, is a great simplification, but it is very close to the nest maps commonly used in studies of wasps.

Our analyses were based on dry nest collection, but the use of two-dimensional nest maps permits the combination of data from living (e.g. field observations) and collected nests. Recording a nest map requires only some seconds in the field. This record can be a good-quality photograph or a record of the nest outline in a hexagonal grid to recall the two-dimensional simplified structure of the nest. With this simplification, the meaning of the given parameter may change. For example the geometric center calculated from the number and position of ideal identical cells is not the same as the centre of gravity of the three-dimensional natural comb. The method of calculation of the center presented here is not affected by the distribution of brood and meconium, and the size and distribution of young cells.

### *The main effects of maturation*

The age (assigned to four maturation categories established from different products of the brood) and the size (cell number) of the nests were taken as the two key variables in the current analyses. These two parameters are normally correlated because of common causal factors (e.g. the fecundity of the queen). In our case, the *Larval* and the *Premature* nests cover the same size range, suggesting that in these

stages the expansion slows or stops, while maturation continues (West Eberhard, 1969, Jeanne, 1972). The analysis clearly indicates that a given nest can reach its *Mature* stage in a wide range of nest sizes.

In the course of maturation of the colony, the brood influence the structure, e.g. the large larvae trigger the lengthening of their cell and the pupae trigger the cell initiation on the periphery (Downing and Jeanne, 1990, Karsai, 1996). Given these facts, the points at which the different effects become operative comprise important information because these are the points in the colony cycle when transformation of the structure begins to take place.

The most striking effect of maturation was on the height of the nest. Nests of different age groups not only differed in  $H$ , but also suggested different dynamics as a function of nest size. *Embryo* nests increased quickly with cell number and exhibited a definite relationship to nest size. In this phase, there are no large larvae to trigger the lengthening. We assumed that the stimuli which govern the lengthening in this stage come from the structure itself. Computer simulations showed that simple building algorithm, which tries to counterbalance the structural heterogeneities, increase the height of the nests quickly at the beginning of building (Karsai and Péntzes, 1993). In this stage, the number of buildable locations is modest (Karsai and Theraulaz, 1995) and this further promotes a greater increase in height.

Although data from nests of 16 to 20 cells are lacking, there was a sudden increase in  $H$  between the *Embryo* and *Larval* nests without an appreciable increase in the number of cells in this range. A small "jump" can be found between the *Larval* and the *Premature* nests, whose heights differed significantly, in contrast with the fact that they covered the same size range. The definite increase in these groups are supported in our data set by a small *Premature* nest that had only 12 cells (Fig. 7). This nest had a  $H$  similar to those of the others in its age category: it was more than 10 mm larger than the *Embryo* nests with the same cell number. The external reference (not coded in the paper structure of the nest) used by the wasps in these two stages seems to be the large larvae which emerge, by definition, at the beginning of the *Larval* stage. After the third instar, the larvae grow in both diameter and length, and the wasps lengthen the cell if it is not able to enclose the larva. Experiments and direct behavioral observations lend additional support to the triggering effect of the large larvae (Gervet, 1966; Downing and Jeanne, 1990; Karsai, 1996).

The height of a cell (and consequently the height of the comb) generally increases according to the requirements of the larva. After the fifth instar, when the larvae reach their maximum size, further enlargement is not necessary. If the cell is reused after the imago ecloses, only limited further enlargement is necessary to reclaim the depth lost to the meconium of the previous brood. We assume that in the case of *Mature* nests the meconium induces a place limitation only for the fifth instar larvae, which in turn triggers the lengthening behavior. For every consecutive larva in a given position, only a small cell enlargement is necessary (the requirements of the structure and the larva are fulfilled). Overall, this appears as a slight increase in  $H$  as the nest develops.

### Diameter ratio

While the third dimension of the nest ( $H$ ) is under the control of the three different constraints mentioned above, the other two dimensions (the length and width of the comb) are seemingly independent of them. Although the diameter ratio is insensitive to the lobes or other irregularities of the comb, it is able to quantify the main comb forms. As our analysis reveals, this variable is independent of both the size and the age of the nest. It can therefore be a good candidate for use as a species-specific characteristic. It must be born in mind that the diameter ratio may display characteristic changes in several species in the course of nest ontogeny (e.g. Wenzel, 1989).

### Eccentricity

The other candidate for a species-specific characteristic is the eccentricity, which has been used by students of wasps in a slightly different manner. Downing and Jeanne (1986) and Yamane (1984) treated the eccentricity of combs only semiquantitatively. Wenzel (1989) used circular statistics, and was able to test whether a given nest was eccentric or not. The geometrical center was defined visually in these studies and the applied statistical method does not work for small nests because of the condition of  $\chi^2$  test. The method we have described involved an exact calculation of the center and gives a geometric distance between this center and the petiole of every nest.

Detailed studies of the behavior of the individuals show that the positions of new cells are determined most often by the egg-laying female (Downing and Jeanne, 1987; Pratte, 1990). This is particularly true in the early stages of the colonies, when there are no or only few workers. Later, in the *Mature* phase, more builders (and consequently more workers) initiate the new cells, it may result in differences in structure. Wenzel (1989) described a similar phenomenon in *P. annularis* but in that species the trend was the opposite of ours, i.e. early constructions were more eccentric.

Theoretical models show (Karsai and Péntzes, 1993; Péntzes and Karsai, 1993) that a well-centered structure with a small eccentricity can emerge from simple building rules which are based only on local cues. The simulated wasps, which have no concept of the shape of the comb and the locality of the first cell, used only immediate and one-cell magnitude cues for the construction, but were able to build well-centered nests.

The diameter ratio and eccentricity are not believed to be independent from each other. Elongated nests generally, have an eccentric and inclined character, while round combs are neither inclined nor off-centered. The eccentric character has been ascribed to the angle of inclination since *P. instabilis* and *fuscatus* tend to build by adding cells to the lower edge of the comb (Downing and Jeanne, 1987). Wenzel (1989), studying the nest of *P. annularis*, found relationships between the inclination and the eccentricity of the nests, although the correlation was not very strong. Our results on the two-dimensional framework partly agree with those findings: the nests of *P. dominulus* are round, well-centered, and the comb is



horizontal (not inclined). However, on an intraspecific scale there was no significant correlation between the diameter ratio and the eccentricity.

### *Size-dependent variables*

The shapes of the combs of *P. dominulus* were round, and it therefore seemed reasonable to base an analysis on regular round combs. Although the calculation of cell number for idealized growth has been traditional, at least since Saussure (1852–1858), some of these analyses contain faults (Ishay et al., 1982) or analyze only a specific problem (Wenzel, 1989). The ideal structures can be used as a reference to study the shapes of real combs. The numbers of outer walls and buildable cells did not differ significantly from those calculated for ideal growth. The compactness, a variable which is more sensitive to the differences in the cell arrangement, was only able to detect the slight irregularities of the natural structures. The compact comb not only has advantages because of the more economic material usage (see Jeanne, 1975; Ishay et al. 1982), but also seems to be important for maintenance of the social cohesion of the colony (Starr, 1991).

### *Structure and behaviour*

Cell initiation takes place on the circumference of the comb, where at least two older cells being used to support the new cell. In *P. dominulus*, generally two ( $B_2$ ) or three ( $B_3$ ) older cells contribute to the walls of the new cell. Completion of a new cell in an  $B_3$  position decreases or maintains the value of  $B_3$ . On the other hand, building on an  $B_2$  position often increases the number of  $B_3$  by two units. In *P. dominulus*, it becomes apparent that the number of  $B_3$  positions is smaller than would be expected from random initiation (Pérez and Karsai, 1993). This means that the wasps do not initiate the cells randomly. During the nest growth, builders keep this value small. It is possible, as observations on the initiation behavior indicate (Downing and Jeanne, 1990; Karsai and Theraulaz, 1995), that the wasps prefer to complete the three-walled cells in the initiation, rather than to begin a new row or initiate a lone cell somewhere. Theoretical studies revealed that the strong but not exclusive preference for the initiation of new cells in an  $B_3$  position is sufficient to keep the  $B_3$  at a low level, besides it produces compact and well-centered combs (Karsai and Pérez, 1993; Pérez and Karsai, 1993).

The low number of  $B_3$  positions suggested different dynamics in the different age phases. After the *Embryo* stage, where the maximum of  $B_3$  was only one, a sudden jump was observed. On the other hand, in the *Larval* and *Premature* nests, this value did not vary with the nest size: it remained between 0 and 7. These phenomena suggest that there is a switch in the stimuli that affects the position of initiation. While the stimuli which govern the initiation in the *Embryo* stage may stem from the nest structure (Pérez and Karsai, unpublished), in more developed nests they stem from the brood (Karsai, 1996). In the *Mature* phase, the random searching can give a good explanation for the slight increase in  $B_3$  with the nest size, because the builder has less chance in a large comb to find these positions during its ritualized random searching.

### Quantitative traits

In this study, we set out to attain a quantitative description of the intraspecific variation in the nests of *P. dominulus*. Several variables used earlier (e.g. wall expenditure and diameter ratio) were reexamined and a number of new ones were suggested. Depending on the aim of a given study, it is reasonable to choose those suggested parameters that are able to express the variability of the structures and are easy to calculate (see, for example, the different parameters describing different properties of cell arrangements). For comparisons within interspecific or intergeneric levels, it seems appropriate to use the more robust diameter ratio and the wall expenditure. For intraspecific studies, the slopes of fittings of the outer walls or the compactness parameters are recommended.

Quantitative nest traits are not only useful as phylogenetic or taxonomic characters, but also permit detection of the structural changes in the nest due to given behavior. In practice, it would be reasonable to describe the structures in a standardized quantitative way which would allow immediate comparisons and further calculations.

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## Appendix I<sup>1</sup>

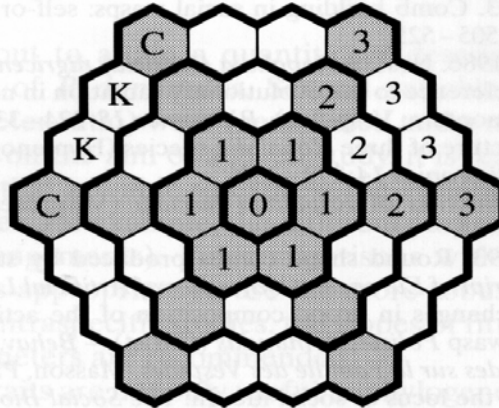
### Cells:

In a regular comb, the structure is composed of completed rings ( $r=0, 1, \dots, R$ ), where the first ring ( $r=0$ ) is composed of one cell. The size of the comb can be expressed in terms of the number of rings ( $R$ ) (Fig. 8).

The rings consists of regular hexagonal cells. Two cell types can be distinguished within a ring:

- corner cells situated at the crossing points of the rows; the number of corner cells in every ring in 6 (from the 6-order center of symmetry);

<sup>1</sup> Detailed computation sheet and simple basic program for calculating Compactness and Eccentricity variables are available upon request.



**Figure 8.** The regular nest. Numbers denote the number of rings. Grey cells and C: corner cells; white cells and K: row cells. Thick lines: external walls ( $S_e$ ) of cell ring  $r$  and internal walls ( $S_i$ ) of ring  $r+1$ ; thin lines: ring walls ( $S_r$ )

– row cells situated between two corner cells forming a cell row; the number of row cells in any ring  $r$  is  $6 \cdot (r-1)$ .

The number of cells in a given ring  $r$  is  $N = 6 \cdot (r-1) + 6 = 6 \cdot r$ .

The total number of cells ( $N$ ) in a whole comb composed of  $R$  rings is (see also expression (1)):

$$N(R) = 1 + 6 \cdot \sum_{r=1}^R r = 1 + 3 \cdot R \cdot (R+1).$$

**Walls:**

Each cell has 6 walls, but two adjacent cells share a common wall. For a given ring  $r$ , three wall types can be distinguished according to their position relative to the adjacent rings (Fig. 8):

- Internal walls ( $S_i$ ) are shared with ring  $r-1$  of the comb.
- External walls ( $S_e$ ) are shared with ring  $r+1$  (if it exists:  $r \leq R$ ).
- Ring walls ( $S_r$ ) interconnect the cells constituting the same ring.

The numbers of different wall types for closed rings are as follows:

- corner cells:  $S_i$ : 1;  $S_e$ : 3;  $S_r$ : 2;
- row cells:  $S_i$ : 2;  $S_e$ : 2;  $S_r$ : 2.

The numbers of different wall types in ring  $r$  are

$$\begin{aligned} S_i: & 6 + (r-1) \cdot 6 \cdot 2 = 6 \cdot (2 \cdot r - 1); \\ S_e: & 6 \cdot 3 + (r-1) \cdot 6 \cdot 2 = 6 \cdot (2 \cdot r + 1); \\ S_r: & (r \cdot 6 \cdot 2) / 2 = 6 \cdot r. \end{aligned}$$

The wall contribution of a new ring  $r$  to the comb is  $S_e + S_r = 6 \cdot (3 \cdot r + 1)$ .

The number of outer walls ( $S$ ) and the total number of walls ( $Z$ ) in a comb consisting of  $R$  rings are

$$S(R) = S_e = 6 \cdot (2 \cdot R + 1);$$

$$Z(R) = 6 + 6 \cdot \sum_{r=1}^R (3 \cdot r + 1) = 6 + 3 \cdot R \cdot (3 \cdot R + 5).$$