

# Developmental and Dominance-Associated Differences in Mushroom Body Structure in the Paper Wasp *Mischocyttarus mastigophorus*

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**ABSTRACT:** Primitively eusocial paper wasps exhibit considerable plasticity in their division of labor. Dominance interactions among nest mates play a strong role in determining the task performance patterns of adult females. We asked whether dominance status and task performance differences were associated with the development of subregions of the mushroom bodies (MB) of female *Mischocyttarus mastigophorus* queens and workers. We found that the MB calycal neuropils were better developed (relative to the Kenyon cell body layer) in the dominant females that spent more time on

the nest. Increased MB calyx development was more strongly associated with social dominance than with high rates of foraging. The MB of queens resembled those of dominant workers. The results suggest that social interactions are particularly relevant to *M. mastigophorus* females' cognition. By examining the MB of newly emerged females, we also found evidence for significant age-related changes in MB structure. © 2006

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## INTRODUCTION

Division of labor is a fundamental feature of insect societies. A diversity of tasks, including egg laying, brood care, nest maintenance, defense, and foraging, must be performed simultaneously by the workers and reproductives (queens) to achieve colony fitness. Because nest mates specialize by performing different tasks, eusocial insects provide an excellent opportunity to analyze adaptive, behaviorally relevant brain plasticity.

The mushroom bodies (MB) of insect brains have received increasing attention from neuroethologists because of their roles in sensory integration, learning, and memory (Connolly et al., 1996; Heisenberg, 1998; Strausfeld et al., 1998). Eusocial insects have relatively larger MB, particularly MB calyces, than related solitary species (Howse, 1974). The Kenyon cells are the intrinsic neurons of the mushroom bodies, and are believed to participate in multimodal sensory integration and learning. The Kenyon cell bodies form a layer that surrounds the underlying cup-shaped neuropil of the calyces, in which the Kenyon cell dendrites arborize. Kenyon cells receive much of the sensory afferent projections to the MB, including synaptic input from the antennal and optic lobes (Mobbs, 1982; Strausfeld et al., 1998; Ehmer and Hoy, 2000; Strausfeld, 2002). Because the calyces contain Kenyon cell dendritic arbors and synaptic input, changes in calycal volume may reflect dendritic growth and synapse addition onto Kenyon cells.

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Research in highly eusocial insects, including honey bees (*Apis mellifera*), carpenter ants (*Camponotus floridanus*), and paper wasps (*Polybia aequatorialis*), links plasticity of the mushroom bodies with division of labor among social insect workers (Withers et al., 1993; Gronenberg et al., 1996; O'Donnell et al., 2004). Workers of many highly eusocial insects, such as honey bees, ants, and *Polybia* paper wasps, are specialized in their roles as reproductively limited helpers. These workers further specialize by performing different tasks for their colonies. The workers exhibit temporal polyethism, progressing as they age from in-nest tasks to work at the nest periphery, and finally to foraging away from the nest (Winston, 1987; O'Donnell and Jeanne, 1992). As workers move among different tasks, the complexity of tasks and the intensity of stimuli to which they are exposed (for example, light levels) can change markedly. In *P. aequatorialis*, workers' MB calyx to Kenyon cell layer volume ratios increased from in-nest to on-nest to foraging, but the greatest differences were between in-nest and on-nest workers (O'Donnell et al., 2004). This is a pattern expected if increasing light levels are a major determinant of MB calycal development. Honey bee workers' MB calyces increase in volume with foraging experience, suggesting that task complexity can drive brain plasticity (Withers et al., 1993, 1995; Farris et al., 2001).

In contrast to highly eusocial species, the adults of many primitively eusocial species are relatively plastic in their behavior (O'Donnell, 1996). Temporal polyethism is weak or absent in primitively eusocial species. Females can switch among task specializations, and among reproductive roles, in response to changes in the social environment of their colonies. Dominant adults frequently engage in aggressive interactions, and spend much of their time on the nest. Foraging is largely relegated to subordinates (O'Donnell, 1998a). These patterns suggest that the cognitive challenges that confront workers in primitively eusocial species may differ from those facing highly eusocial workers. Social interactions may place greater demands on neural development in primitively eusocial species. More complex social environments induce greater MB calyx development in *Drosophila* fruit flies (Heisenberg et al., 1995), suggesting that the MB are involved in insect social cognition.

The goal of this study was to test two alternative models for the regulation of MB development in primitively eusocial paper wasps. The first is the *task complexity model*, suggested by the data on *Apis* and *Polybia* worker MB development. The task complexity model posits that the cognitive challenges posed by certain tasks, particularly foraging away from the

nest, place greater demands for development of neural circuitry. Foraging could also be associated with greater stimulus intensity such as higher light levels, also leading to increases in brain development. The task complexity model predicts that greater neural complexity will be associated with the performance of certain tasks, particularly foraging. In primitively eusocial paper wasps, subordinate foragers should exhibit the greatest MB calyx development. The second is the *social interactions model*, which is suggested by comparative neuroanatomy (Howse, 1974), by manipulations of social experience in *Drosophila* (Heisenberg et al., 1995), and by data on *Polistes* paper wasp foundress associations (Ehmer et al., 2001). This model posits the rate or intensity of social interactions is the primary cognitive challenge that the animals face. Therefore, the social interactions model predicts that dominant nest wasps will exhibit the greatest MB calyx development.

We quantified differences in MB development among behavioral castes in the primitively eusocial paper wasp *Mischocyttarus mastigophorus*. As in many primitively eusocial paper wasps, division of labor in *M. mastigophorus* is strongly influenced by dominance interactions (Reeve, 1991; O'Donnell, 1998a, 1999; Keeping, 2002; Strassmann et al., 2004). *Mischocyttarus mastigophorus* females leave the nest to collect food and building materials, and subordinates forage at higher rates. Subordinate foragers spend less time on the nest, where dominance and other social interactions occur (O'Donnell, 1998a, 1999). This study is the first assessment of what factors might affect differential MB development in post-emergence, or worker-producing, primitively eusocial wasp colonies.

If the calyces of *M. mastigophorus* foragers were relatively well-developed, this would suggest that the cognitive challenges of off-nest task performance, or the more complex environmental stimuli experienced by foragers, are the primary determinants of MB development. Alternatively, if the calyces of dominant nest wasps were relatively well-developed, this would suggest that social interactions are more important determinants of MB development. The latter pattern would suggest that the major cognitive challenges that face primitively eusocial insect workers differ from those that face workers in highly eusocial species.

## METHODS

Field research was conducted from 20 June to 20 July 2004 in Monteverde, Costa Rica, 10°18'N, 84°48'W. The Monteverde area comprises clearings for homesteads, pastures and research facilities in tropical cloud forest (Nadkarni

and Wheelwright, 2000). *Mischocyttarus mastigophorus* is restricted to elevations above approximately 1450 m (O'Donnell and Joyce, 1999, 2001). All subject wasp colonies were located on the eaves of buildings, at elevations of 1450–1540 m above mean sea level. We used seven post-emergence (adult producing) colonies as subjects.

### Marking, Behavioral Observations, and Collection of Subjects

Starting 1–5 days before behavioral observations, we marked all of the adult wasps on the subject colonies. To mark the wasps, we first collected them into a plastic bag and lightly anesthetized them with Halothane. We marked the wasps on the dorsum of the thorax for individual identification with paint pens, then returned them to their nests as they regained mobility (O'Donnell, 1998a). We marked 7–13 adults per colony (mean = 9.9 adults/colony), of which 7–11 were females (mean = 8.3 females/colony). Adult wasps that emerged during behavioral observations were not marked, but were collected at the end of observations.

Each colony was observed on 2 consecutive days for 8 h total observation time. We collected behavioral data in two continuous 2 h blocks, separated by a break of 30 min–1 h, beginning from 0700 to 0730 h local time each day. During the observation sessions we recorded all occurrences (with time to the nearest min) of foraging departures and arrivals. We also recorded all occurrences of dominance interactions noting the identity of the dominant and subordinate individual. Dominance interactions in *Mischocyttarus* include chasing and biting. The dominant wasp is clearly identifiable in each interaction. Subordinates crouch when bitten, or flee. See O'Donnell (1998a, 1999) for more details on *M. mastigophorus* behavior. To insure that all adults would be present, the wasps were collected after dark (1000 h or later) on the last day of observations. We noted the eye color (pale or dark) of the adult wasps when they were collected. The compound eyes of *M. mastigophorus* adults are dark upon adult emergence, and gradually grow paler as the wasps age. Dark eye color persists for at least several days (O'Donnell, personal observation). The wasps were placed directly into Prefer fixative, (Anatech Ltd., Battle Creek MI), and stored in fixative until histological processing was performed.

We collected the combs of two *M. mastigophorus* nests with capped pupal cells and held them indoors in mesh containers. These were checked daily for adult emergences. Adults from these nests were collected directly into Prefer fixative within 24 h of emergence, and stored in fixative. We also obtained newly emerged adults from some of the observation colonies.

### Behavioral Variables

We quantified the independent relationships of social interactions and of task performance with MB structure. Because many wasps were not observed interacting with each other, we used an index of dominance status to assess female's

social status (O'Donnell, 1998a). The index summarizes each female's relative rate of experiencing dominance and subordination per hour spent on the nest:

$$\text{Dominance index} = \frac{(\text{Number of interactions when dominant}) - (\text{Number of interactions when subordinate})}{\text{Number of observation hours present on nest}}$$

This index is strongly associated with individual differences in task performance and reproductive physiology in *M. mastigophorus* (O'Donnell, 1998a; Markiewicz and O'Donnell, 2001). More dominant wasps forage for food less, and have better developed ovaries. We used this index to calculate each female's relative social rank within her colony.

As an indicator of differences in task performance we used the proportion of observation time each female spent on the nest. The proportion of time spent on the nest was calculated from the foraging arrival and departure data. Time away from the nest is strongly associated with food foraging behavior in *M. mastigophorus* (O'Donnell, 1998a, 1999).

We selected subjects for neuroanatomical analysis based on their behavior. The one female in each colony that was observed laying eggs was designated as the queen. We used the queens, dominant workers, and subordinate workers (high and low values in dominance rank, respectively) as subjects.

### Histology and Neuroanatomical Measures

We collected neuroanatomical data from two to five marked subjects per colony (total  $n = 23$ , mean = 3.3/colony). We also collected neuroanatomical data from five newly emerged unmarked females from observation colonies and laboratory incubated combs. All subjects were adult females. Neuroanatomical data were collected blind to the behavior of the subject.

Wasp head capsules were dissected from the body and embedded in epoxy resin (Embed 812, Electron Microscopy Sciences, Hatfield, PA). The head capsules were opened to improve resin infiltration by dissecting the mandibles away. All embedding was performed in a single batch to ensure consistency of histological processing effects between subjects. The embedding involved an ascending series of ethanol concentrations (50–100%) and acetone:resin concentrations performed on a tissue rotator. Several steps were performed under vacuum to improve infiltration. Heads were positioned individually in plastic molds filled with resin (8 mm diameter embedding capsule caps) and polymerized in a 58°C oven for 3 days. The resin blocks were then mounted on acrylic pegs and cut using a rotary microtome with disposable stainless steel blades at a microtome thickness setting of 10  $\mu\text{m}$ . To verify the thickness of the sections, the notched block method (Korbo et al., 1990) was performed in samples that had been included in the same resin embedding batch ( $n = 21$ ). The distance between the block face and a notch made below the sample was measured with a vernier caliper before

and after sectioning. The average thickness per section was calculated as the difference in block height/number of sections. This yielded an average thickness of  $9.92 \pm 0.17 \mu\text{m}$  (mean  $\pm$  SEM). Therefore, in the calculations  $10 \mu\text{m}$  was used as the average section thickness. Sections were placed in a spot of 20% acetone on gelatin-coated slides, warmed on a hot plate and subsequently stained using Toluidine blue (a Nissl stain). To reduce section rippling, weights were placed on the coverslips until the mounting media dried ( $\sim$ 1–2 weeks).

The MB is divided into a surrounding layer of Kenyon cell bodies, and the calyx, which comprises the underlying neuropil (Fig. 1, Mobbs, 1982). The calyx is further subdivided into anatomically and functionally distinct regions. We focused on the calycal lip, which receives chemosensory antennal input in Hymenoptera, and the collar, which receives visual input from the compound eyes (Ehmer and Hoy, 2000; Gronenberg, 2001; Strausfeld, 2002). The volumes of mushroom body subregions were estimated using the Cavalieri procedure (Henry and Mayhew, 1989) with the aid of ImageJ public domain software (Rasband, 1997–2006). Sections were viewed using an Olympus BX61 microscope. Images of the mushroom bodies of one hemisphere were captured using a high resolution digital camera (DVC Inc., Austin, TX) and viewed at a final magnification of  $800\times$ . A systematic random sampling scheme was used in which the first section sampled was randomly chosen from the first three rostral most sections containing mushroom bodies and thereafter every third section was included in the analysis, moving caudally (Mayhew, 1992). Images were imported into ImageJ and overlaid with a grid of test points. Points identified as falling within lip, collar, and basal ring subregions of the calyces and within the Kenyon cell body region were counted using a point counting plug-in program. Volume was calculated as a product of the number of points counted, summed over sections, the area per point ( $0.0014 \text{ mm}^2$ ) and the distance between section planes ( $30 \mu\text{m}$ ).

## Statistical Analyses

To test for relationships of behavior with MB plasticity, we used the ratio of calyx volume:Kenyon cell body layer volume as the response variable. We used multiple regression (GLM procedure in SAS version 9.1) to first account for colony differences, then to test for relationships of dominance rank and proportion of time on nest with MB structure. We used the ratio of two major MB subregions, the lip and collar, to test for differential development of functionally distinct areas within the MB calyx. We compared total volumes of the Kenyon cell layer and the calyx of adults grouped by age and behavioral caste, pooled across colonies, using multiple regression.

## RESULTS

Dominance rank values were negatively correlated with the proportion of observation time the wasps

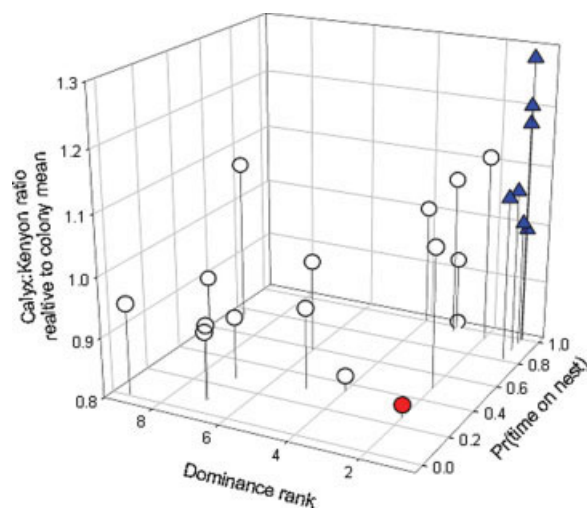


**Figure 1** Representative frontal sections showing the anatomy of the mushroom bodies of a queen, a worker, and a newly-emerged ( $<24$  h old) female of *Mischocyttarus mastigophorus* paper wasps. Regions that were quantified in this study are labeled. K: Kenyon cell bodies; C: collar of the calyx; L: lip of the calyx; BR: basal ring of the calyx (indicated by the arrowhead). Scale bars represent  $100 \mu\text{m}$ . [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

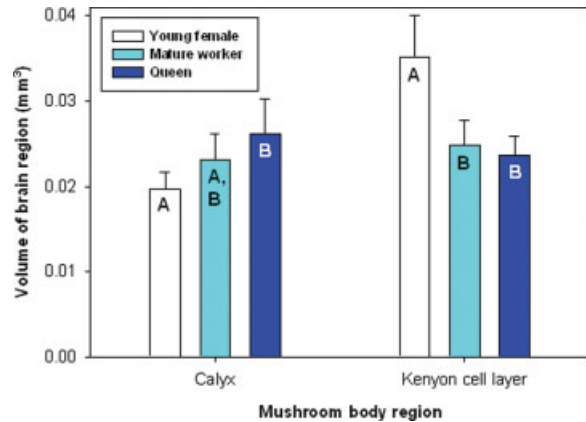
spent on the nest ( $n = 23$ ,  $r = -0.67$ ,  $p = 0.0005$ ). Mushroom body structure was strongly associated with behavior for adult females (Overall model for

calyx: Kenyon cell layer ratio,  $r^2 = 0.62$ ,  $p = 0.039$ ). The ratio of total calyx volume to Kenyon cell layer volume increased positively and independently with both dominance rank (Fig. 2;  $F_{1,14} = 4.86$ ,  $p = 0.045$ ) and with the proportion of time the wasp spent on the nest (Fig. 2;  $F_{1,14} = 6.25$ ,  $p = 0.026$ ). The queens had the highest dominance ranks, and also ranked high in time spent on nest, but their calyx:Kenyon cell ratios often overlapped those of dominant workers (Fig. 2). MB subregions (lip and collar) did not differ in their relationships with behavior (Overall model for lip:collar ratio,  $r^2 = 0.45$ ,  $p = 0.27$  NS).

Comparisons of newly-emerged adult females with older females suggest that strong changes in MB structure occur during adult development. Pooled across colonies, age and caste categories differed in calyx volume ( $F_{2,24} = 5.81$ ,  $p = 0.009$ ) and in Kenyon cell layer volume ( $F_{2,24} = 22.38$ ,  $p < 0.0001$ ). Post hoc comparisons showed that queens and workers did not differ in either measure, while young females differed from queens in calyx volume and from both queens and older workers in Kenyon cell layer volume (Fig. 3). MB maturation appears to involve both a large reduction in Kenyon cell layer volume, and a more modest increase in calyx volume. The single dark-eyed marked female had a calyx:Kenyon cell body ratio that was lower than expected



**Figure 2** 3D scatter plot showing the relationship between mushroom body structure (calyx:Kenyon cell body volume ratio) and behavior for female *Mischoxyttarus mastigophorus* paper wasps. Behavioral variables are dominance rank and proportion of time on nest. Queens are represented by shaded triangles, workers by open circles. A young (dark-eyed) female is indicated by a shaded circle. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]



**Figure 3** Age and caste differences in the size (volume) of mushroom body regions in female *Mischoxyttarus mastigophorus* paper wasps. Bar heights represent mean  $\pm$  SD. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

for her social rank (Fig. 2). This female was at least six days old when she was collected.

## DISCUSSION

Females of tropical primitively eusocial paper wasps such as *M. mastigophorus* are notably plastic in their behavior, and their reproductive division of labor is dynamic. At any point in the colony cycle, females can ascend dominance hierarchies to inherit their natal nests, or help to initiate new colonies, often with groups of nest mates (Chandrashekhara and Gadagkar, 1992; Sinha et al., 1993; O'Donnell and Joyce, 2001).

*Mischoxyttarus mastigophorus* are excellent subjects for the analysis of brain plasticity and its relationship with individual behavioral differences. New *M. mastigophorus* colonies are initiated throughout the year in the Monteverde area (O'Donnell and Joyce, 2001). Therefore, a newly emerged female's reproductive caste status is not seasonally constrained, as it is for many temperate paper wasp species (O'Donnell, 1998b; Hunt et al., 2003). Furthermore, females' task performance patterns depend on their dominance status, and their behavior can change in response to changes in colony social structure (O'Donnell, 1998c).

Our data suggest that a female's position in her social hierarchy is related to brain development, particularly development of the MB calyx. The dominant nest wasps, including queens and workers, had greater calyx:Kenyon cell body volume ratios than foragers. Dominant females engage in social interactions at high rates, in part because they spend more time on the nest than subordinate foragers. The dominance-related

patterns of brain development suggests that social interactions have special cognitive relevance to adult female *M. mastigophorus*.

There may be a general brain development-dominance behavior relationship in primitively eusocial paper wasps. Ehmer et al., (2001) found similar patterns when they compared solitary and social nest founding groups of *Polistes dominulus* paper wasps. The females in social groups had larger antennal lobes and MB collars than the solitary nesting females, suggesting that social interactions played a strong role in affecting MB and antennal lobe development. Because the solitary females cannot rely on division of labor, they need to perform a wider range of tasks. Therefore, foraging and other tasks do not explain brain development. These results are comparable to our findings that foraging and other tasks performed by subordinate wasps is not the major determinant of MB development.

Within the *Polistes* foundress social groups, dominant and subordinate foundresses did not differ in brain development, though their task performance rates were not measured (Ehmer et al., 2001). In further contrast to the data on *Polistes* cofoundresses, we did not find evidence for differential development of the MB calyx lip and collar regions, which receive primarily chemosensory and visual input, respectively, in other eusocial Hymenoptera (Gronenberg, 2001; Strausfeld, 2002). A diversity of sensory modalities are likely to be used during social interactions in paper wasp colonies. Low-volatility pheromones, including cuticular hydrocarbons, are perceived by the antennae and are important in paper wasp social recognition (Panek et al., 2000; Dapporto et al., 2004). Tibbetts and Dale (2004) recently demonstrated that *Polistes* females can respond to visual cues of social rank as well. A general increase in cognitive capacity, distributed among brain regions, may be necessary for dominant females.

We also showed that the MB of female *M. mastigophorus* undergo developmental reorganization following adult emergence. The fact that a young female with dark eyes that was marked on the nest had MB structure that was similar to newly emerged wasps suggests that the reorganization may take several days, but we could not precisely determine the time course. The pattern of expansion of the calyx and reduction in the Kenyon cell body layer suggests that increases in arborization may be supported by the transfer of cytoplasm into the calyx. In *Apis*, foraging causes increased dendritic branching of Kenyon cells in the MB collar, which may contribute to the volume expansion of the calyx (Farris et al., 2001). However, we did not quantify Kenyon cell changes at the neuron level, and reductions in MB intrinsic neuron densities,

or changes in the packing density of Kenyon cell bodies, are other possible explanations for the volume changes that we observed. MB calycal expansion does not involve neurogenesis in honey bees (Fahrbach et al., 1995).

Our finding that the MB calyces of subordinate foragers were smaller than the calyces of dominant nest wasps is important because it contrasts with results on honey bee and *Polybia* paper wasp workers (Withers et al., 1993, 1995; Durst et al., 1994; O'Donnell et al., 2004). In both *Apis* and *Polybia*, which are highly eusocial species, the MB calyx:Kenyon ratios are greater in foragers than in nest workers (Farris et al., 2001; O'Donnell et al., 2004). This pattern has been interpreted as reflecting the greater cognitive demands associated with foraging (Fahrbach and Robinson, 1996), and possibly with higher light levels and other stimuli outside the nest.

We did not measure the body size of the subjects. The behavior associated MB volume differences that we tested were within-individual ratios, and would not be affected by body size. The caste and age comparisons of MB region volumes could have included a body size effect. In bumble bees workers and *Polistes* paper wasp nest founders, brain size in general, and MB volume in particular, correlates positively with body size (Ehmer et al., 2001; Mares et al., 2005). In primitively eusocial paper wasps, the behavior of females that found new nests together is often affected by body size, with larger females tending to dominate smaller (Pratt, 1989; Keeping, 2000; Fukuda et al., 2003). However, we do not expect body size effects to be important in our study because previous work on *M. mastigophorus* found no relationships of body size with behavioral or physiological variables (Markiewicz and O'Donnell, 2001).

Because we did not know the ages of the marked subjects in the study, we cannot assess the relative importance of age and task experience in explaining MB development in *M. mastigophorus*. Longitudinal observations on individually marked, known-age females will be necessary to quantify age effects. In honey bees and carpenter ants, MB calycal expansion is associated with foraging, but a general increase in calyx volume with age also occurs (Gronenberg et al., 1996; Fahrbach et al., 1998). However, it is clearly not the case that foraging is associated with the most enhanced MB development in adult *M. mastigophorus* females.

*Mischocyttarus mastigophorus* males present a particularly interesting comparison to females. The males are long-lived, reside on their natal nests, and participate extensively in social interactions. Males are dominant over females and control much of the food flow into *M. mastigophorus* colonies. These patterns

are rarely observed among eusocial Hymenoptera (O'Donnell, 1999). Given the high rate of male social interactions, we predict that patterns of development and behavior-associated variation in MB structure in *M. mastigophorus* males will resemble those of females. In particular, we predict that the MB calyces will be better developed in more dominant males.

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