Morphology and Chemical Contents of Dufour Glands of *Pseudomyrmex* Ants (Hymenoptera: Formicidae)

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The morphology and ultrastructure of the Dufour gland of a pseudomyrmecine ant is described. The gland has an unusually large volume but possesses a very thin epithelial lining. Its morphology does not constitute a specific subfamily character as in some ant subfamilies. Analysis by gas chromatography-mass spectrometry of the glands of three species showed them all to be rich in saturated linear hydrocarbons ($C_{17:0}$ and $C_{15:0}$ dominant) and isopropyl esters of the common fatty acids. Each species produces its own characteristic mixture of these substances. The most abundant ester in *Pseudomyrmex ferruginea* is isopropyl oleate and in *P.* sp. A it is isopropyl palmitate, in *P. flavicornis* there is only a trace of isopropyl palmitate. An alate female of *P. ferruginea* contained a very similar mixture to that of the workers.

Introduction

The Pseudomyrmecinae form a small ant subfamily with a pantropical distribution range, comprising approximately 300 species. Members of this group are most renowned because of their intimate relationship with myrmecophytes, in which they find suitable nesting places, and which provide them with food in the form of nectaries or Beltian bodies. The plants, in turn, receive through the presence of the ants, effective and vital protection from herbivores and the competition of other plants growing in close proximity [1, 2]. The ants behave extremely aggressively toward any kind of intruder into this habitat. For this purpose, they use a very well developed sting apparatus, the venom of which causes a severe burning reaction in its victims.

The genus *Pseudomyrmex* is limited to South and Central America and the southern United States, where it lives as an obligatory host in *Acacia* trees. These species possess a powerful sting, into which open the poison gland and Dufour gland, as is the normal situation for all stinging ants. The poison gland in *Pseudomyrmex* contains a proteinaceous secretion [3] with a polysaccharide fraction which has been well studied [4]. No chemical data dealing with the Dufour gland contents in the Pseudomyrmecinae are available so far, except for a very preliminary report of pentadecane as its major constituent [5].

Morphological information on the pseudomyrmecine sting apparatus is limited to a very general anatomical description of the poison and Dufour glands in *P. pallidus* [3], and a brief ultrastructural report on the Dufour gland in *P. termitarius* [6].

The present paper describes a light and electron microscope study of the Dufour gland in workers of *P. ferruginea,* and at the same time provides a first detailed chemical analysis of this gland in three species of this formicid subfamily.

Materials and Methods

For morphology, either abdominal halves or excised single Dufour glands of foraging workers from laboratory colonies of *Pseudomyrmex ferruginea* originally collected in Mexico, were fixed in 2% glutaraldehyde, buffered at pH 7.3 with 0.05 M sodium cacodylate. After postfixation in 2% osmium tetroxide and dehydration in a graded acetone series, tissues were embedded in Araldite and sectioned with a Reichert Ultracut microtome. Semithin sections for light microscopy were stained with methylene blue and thionin. Thin sec-

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tions for electron microscopy, double-stained with uranyl acetate and lead citrate, were viewed in a Philips EM 400 microscope.

For chemical analysis, foraging workers of *P. ferruginea* (F. Smith), *P.* sp. A (near *veneficus,* P. S. Ward, pers. comm.) and *P. flavicornis* (F. Smith) (corresponds with the former name *belti,* P. S. Ward, pers. comm.) from laboratory colonies originating from Central America were killed by nitrogen cooling, their Dufour glands dissected under water, dried by touching with a paper tissue and sealed in soft glass capillaries (20 × 1.8 mm) as described in earlier papers from our laboratory. The sealed capillaries were placed in the injector area of the gas chromatograph, and after two minutes heating, were crushed in the device described by Morgan and Wadhams [7] to introduce the volatile material onto the column without the intervention of solvents. The gas chromatograph was coupled to a mass spectrometer for identification of the separated components. The work on *P.* sp. A was performed on a Hewlett-Packard 5890 Gas Chromatograph and 5970 B Mass Selective Detector with HP 59970 C Chem Station for data handling. A fused silica capillary column (12 m × 0.2 mm) coated with HP-1 of 0.33 μm film thickness was used. Helium was the carrier gas at 1 ml min^{-1}. The oven temperature was programmed from 60 °C at 4 °C min^{-1} to 250 °C. The other two species were examined with a Finnigan 3200 E quadrupole spectrometer with a 6000 Data System. For *P. ferruginea* a fused silica column (CP-19, 38 m × 0.22 mm) was used with helium at 1 ml min^{-1} and a temperature programme from 130 °C to 210 °C at 3 °C min^{-1}. For *P. flavicornis* an SP 2340 column (25 m × 0.22 mm) was used, the oven temperature was held at 60 °C for 4 min, then programmed at 4 °C min^{-1} to 195 °C and then held for 15 min.

n-Propyl and isopropyl fatty acid esters were prepared as model compounds by heating the appropriate alcohol and acid (total volume 500 μl) together with a drop of conc. sulphuric acid in a V-vial (Wheaton Scientific) at 120 °C for 12 h.

Results

Morphology and ultrastructure

The Dufour gland in *P. ferruginea* appears as a long and slender tube, with a length of up to 350 μm and a mean diameter around 50 μm. It opens into the sting base, on the ventral side of the poison gland duct (Fig. 1).

The epithelial wall of the gland is characterized by its thickness, which ranges between 0.5 and 2 μm in all individuals examined (Fig. 2). Also nuclei have acquired a flattened shape because of the extremely thin epithelium. Cytoplasmic organelles are quite rare, apart from some randomly distributed small mitochondria. Numerous free ribosomes occupy most of the cell volume and thus give the cytoplasm a rather granular appearance. A thin cuticular layer of 0.1 to 0.2 μm thickness lines the epithelium at the lumen side. The greater part of the apical plasma membrane shows a simple topography, with only occasional microvillar differentiations. A distinct basement membrane with only a few isolated muscle fibres surrounds the gland at the hemocoel side (Fig. 2).

In its duct region, the Dufour gland assumes a much flattened shape dorsoventrally. An extensive supply of muscle fibres insert on both sides of the slit-like opening (Fig. 1). In this region, the cuticular lining attains a thickness of about 0.6 μm. The duct cells more or less retain the same thickness as in the gland part, although inserting muscles deeply penetrate between them (compare Fig. 2 and 3, which are at the same magnification). Duct cell nuclei occur in the basal cell halves, that are wedged in between the muscle fibres (Fig. 3). Bundles of microtubules extend from the basal to apical cell membrane in the duct cells. Another structurally similar muscular attachment area is observed for the poison gland duct, although only its ventral wall is involved (Fig. 1).

The ducts of both the Dufour and poison gland open separately on top of a tissue bulb that projects into the sting base. Immediately behind this region, four valves are situated, two from each of the ventral sting lancets (Fig. 4). The sting itself has a total length of approximately 1 mm, which is about half the length of the gaster.

Chemical contents

The mixtures of compounds found in the three species examined here were similar in nature, but their proportions showed species-specificity. The two major compounds in *P.* sp. A Dufour glands (70% of the total) were pentadecane (3, Fig. 5) and

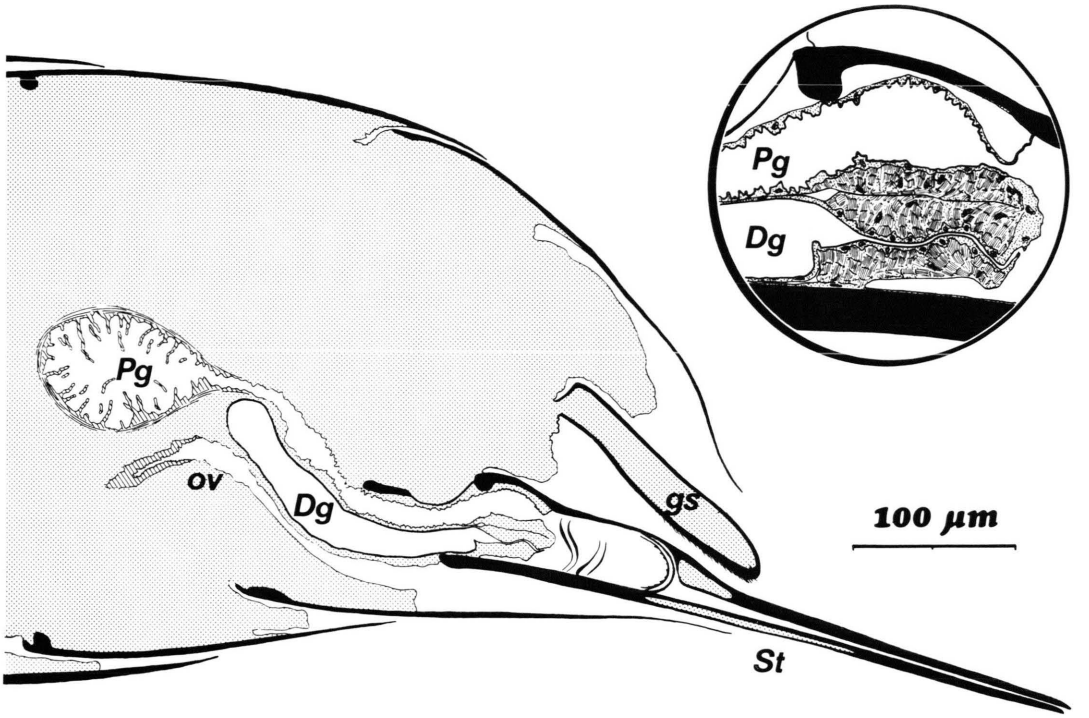
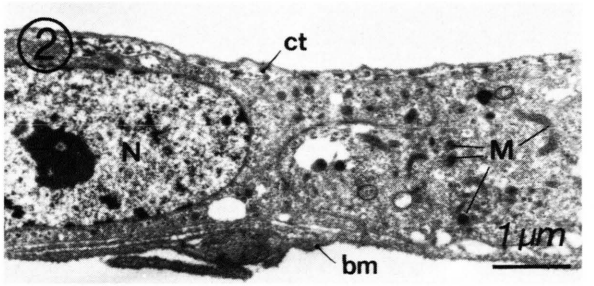
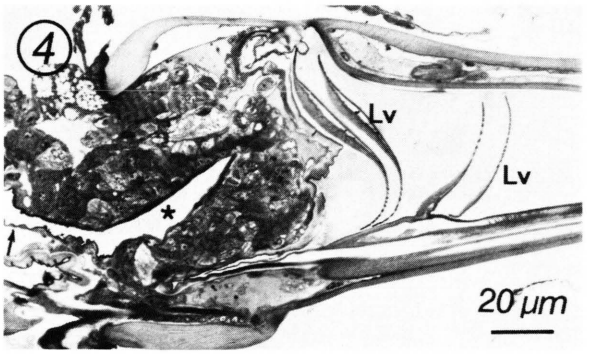


Fig. 1. Schematic drawing of a histological section, showing the structural relationship between the Dufour gland (Dg) and poison gland (Pg), and the sting (St) of *Pseudomyrmex* sp. The entire sting is about twice as long as drawn. gs = gonostylus, ov = oviduct. Inset: detail of muscular supply surrounding the gland ducts in the sting base region.





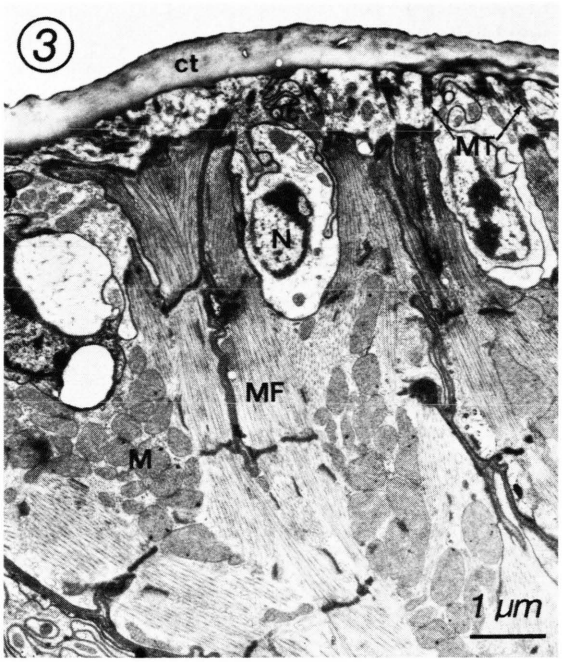


Fig. 2. Electron micrograph of the Dufour gland epithelium. bm = basement membrane, ct = cuticle, M = mitochondria, N = nucleus.

Fig. 3. Electron micrograph of the muscular attachment region (ventral part Dufour gland duct). ct = cuticle, M = mitochondria, MF = muscle fibres, MT = microtubules, N = duct cell nuclei.

Fig. 4. Semi-thin longitudinal section through the sting base, with lancet valves (Lv) and Dufour gland duct (*). Note very thick continuation of the gland epithelium at left (arrow).

heptadecane (7, Fig. 5). Isopropyl palmitate and three other minor components, together with the first two brought the total to 93%. Nineteen trace components, listed in Table I were also identified in some or all of the 11 samples examined. One queen from the colony had the same mixture of substances, in very similar proportions and quantity, so the details are not reproduced here.

Glands of workers of *P. ferruginea* contained three major components, pentadecane, heptadecane and isopropyl oleate (Fig. 6), with six minor components and a total of 23 compounds identified (Table I). There were insufficient samples available to carry out a quantitative measurement of mean amount and percentage as we would wish to do, in order to describe the secretion adequately.

Glands of *P. flavicornis* contained mixtures where the hydrocarbons heptadecane, pentadecane and nonadecane are predominant (Fig. 7). The most abundant ester, isopropyl palmitate, was found only as a minor component. Searching the mass spectral data for other isopropyl esters gave evidence of isopropyl palmitoleate, stearate and oleate at barely detectable levels. Insufficient samples were available for accurate quantitation.

Table I. Substances identified in the Dufour glands of three species of *Pseudomyrmex* by gas chromatography – mass spectrometry.

Substance	Designations	*P.* species A[1] Amount [ng]	SD	%	SD	*P. ferruginea*[2] [%]	*P. flavicornis*[3] [%]
Hydrocarbons							
Tetradecane	1	2.0	1.7	0.3	0.1	0.4	–
Pentadecene	2	1.2	0.8	0.3	0.1	0.3	–
Pentadecane	3	128	123	29.4	4.2	24.0	19
Hexadecane	4	8.6	7.3	2.1	0.4	3.5	t
Heptadecadiene	5	2.6	1.3	0.9	0.6	0.6	–
Heptadecene	6	11.6	10.6	2.7	0.6	9.4	3.9
Heptadecane	7	165	133	43.0	9.4	21.7	73
Octadecane	8	1.7	1.3	0.4	0.2	–	t
Nonadecene	9	t	–	–	–	t	t
Nonadecane	10	11.2	8.5	2.9	0.9	2.2	4.1
Heneicosene	11	1.3	1.4	0.5	0.2	0.7	–
Heneicosane	12	1.2	0.8	0.4	0.3	0.4	–
Tricosane	13	2.2	2.5	0.7	0.4	–	–
Esters							
Isopropyl laurate	A	–	–	–	–	0.2	–
Isopropyl myristate	B	3.6	4.0	0.7	0.2	0.4	–
Isopropyl pentadecanoate	C	2.0	1.5	0.5	0.3	–	–
Isopropyl palmitolenate	D	–	–	–	–	0.9	–
Isopropyl palmitoleate	E	4.1	5.1	0.9	0.5	1.9	t
Isopropyl palmitate	F	62.3	106.1	11.2	10.4	4.9	1.0
Isopropyl linoleate	G	1.4	0.6	0.8	0.4	3.5	–
Isopropyl oleate	H	6.5	7.9	1.1	0.6	23.0	t
Isopropyl stearate	I	1.4	1.1	0.3	0.1	0.3	t
Methyl palmitate	J	t	–	–	–	t	–
Ethyl palmitate	K	t	–	–	–	–	–
2-Butyl palmitate	L	4.0	5.4	0.8	0.3	0.9	–
Others							
2-Pentadecanone	a	t	–	–	–	0.7	–
Tetradecanal	b	1.1	0.1	0.2	0.1	0.2	–
Pentadecanal	d	1.3	0.9	0.3	0.1	–	–

[1] Average of 11 samples. [2] Average of 3 samples. [3] Total of 7 glands. t means trace constituent.

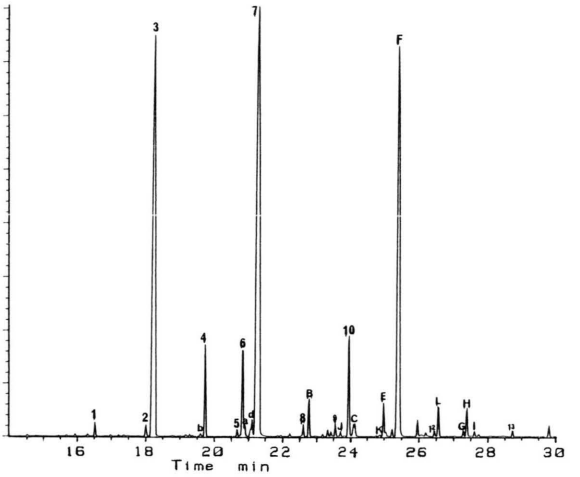


Fig. 5. Example of a gas chromatogram of a single Dufour gland of *P.* species A worker on the HP-1 coated column. For identification of peaks, see Table.

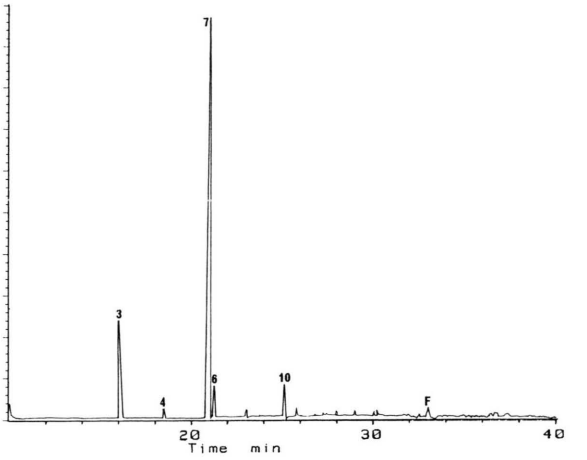


Fig. 7. Gas chromatogram obtained from 5 glands of workers of *P. flavicornis* on the SP 2340 column. For identification see Table.

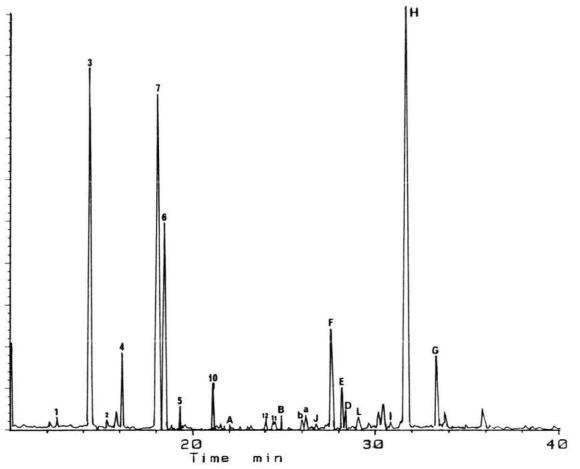


Fig. 6. Gas chromatogram of a Dufour gland of *P. ferruginea* worker, on the CP-19 column. See Table for identification of peaks.

Discussion

The tube-shaped Dufour gland in *P. ferruginea* is most characterized by its extremely thin epithelial wall, the thickness of which varies around 1 μm. Over 60 species belonging to all major ant subfamilies have been examined so far [6], but in no other species has such a uniformly thin epithelium been found. In *P. termitarius,* the only other pseudomyrmecine species so far studied morphologically, a glandular epithelium of 5 μm thickness was found [6], while in *P. pallidus* an epithelium to 15 μm was found (unpublished observations).

Because of its much reduced volume, the Dufour gland epithelium in *P. ferruginea,* at the ultrastructural level, shows a rather poor cytoplasmic composition. A few small mitochondria are observed, while free ribosomes are fairly abundant. A well-developed smooth endoplasmic reticulum, which is a major cytoplasmic element in the Dufour gland of most ants [6, 8], was not observed. There are infrequent microvillar differentiations of the apical cell membrane, but they do not display the typical local clusters reported in *P. termitarius.* Hence it appears that the Dufour gland morphology in pseudomyrmecine ants does not constitute a specific subfamily character, as it does for the Dorylinae, Ecitoninae, Formicinae and Myrmeciinae [6, 9].

According to Blum and Callahan [3], the Dufour gland in *P. pallidus* remains turgid after the act of stinging, whereas the poison gland collapses after secretion of venom. The independent secretion mechanism of both sting glands is clearly related to the muscular organization in their duct region. Both ducts are supplied with a different set of muscle fibres, that exert a pulling force onto the

thickened cuticular lining of the gland duct. In this way, muscular contractions cause the slit-like duct to open, while closure is a passive result of the thickened cuticle resuming its rest position [6, 10]. Although the Dufour gland apparently does not contribute to the real venom production, its contents are also secreted through the sting. During this process, the valves which project up into the sting bulb, will pump secretion out by the reciprocating movement of the lancets.

The total overall volume of the Dufour gland in *P. ferruginea* is in contrast to its thin epithelial lining. Blum and Callahan [3] noted the large volume of the gland to *P. pallidus*. It is difficult to attribute substantial secretory activity to such an extremely thin epithelium, therefore the origin of the glandular secretion is uncertain. Biosynthesis in the haemolymph and subsequent transport through the epithelium into the lumen may be one possibility, although the simple topography of both the basal and apical cell membranes do not much favour this idea. Probably the epithelial wall is more pronounced and metabolically more active at an earlier stage of adult life, and the gland fills slowly for the secretion to be used later. We have shown that in *Formica sanguinea,* adult workers emerge with empty Dufour glands, and it takes up to one year for them to fill completely with the final composition of oily substances [11]. This once again raises the question of function of the Dufour gland, which remains completely unknown for the great majority of ants.

The large volume of the gland found by microscopy corresponds to the microgram quantities of secretion found by chemical analysis. Quantitative results are given in detail for *P.* sp. A only, because only a few samples were available for the other two species, insufficient for good statistical treatment. It will be seen from the table that the *amount* of substance in the glands varies widely (large sample standard deviations) but the average *percentage* composition is rather constant (small sample standard deviations), a situation we have frequently encountered in similar investigations [12]. The average percentage compositions are obtained by calculating the percentage composition for each individual ant and taking the mean values for each compound.

Early work on *Pseudomyrmex* volatiles has shown pentadecane and heptadecane in the Dufour glands of five species [15] and more recently isopropyl esters were found in hexane cuticular washes of *P. ferruginea* [13]. Our present work suggests that the latter originated from the Dufour gland, and explains why the esters were found only in some foragers and not in others. Cuticular washes were contaminated by Dufour gland secretion. However, our *P. ferruginea* sample contained isopropyl oleate, not palmitate, as the major ester.

The predominance of saturated linear hydrocarbons and isopropyl esters in this genus distinguishes it from other ants studied so far, and may prove to be characteristic of the subfamily Pseudomyrmecinae. Esters are frequently found in ant secretions, especially in formicine Dufour glands, but isopropyl esters are rare in insects and have not been found before in ants. Isopropyl esters of C_{12} to C_{18} fatty acids have been found in the male pheromone bouquet of the hide beetle *Dermestes maculatus* [14] and esters of C_{10} to C_{19} fatty acids in the defensive secretion of the rove beetle *Coprophilus striatulus,* where they are described as wetting agents [15].

We prepared *n*-propyl and isopropyl esters of several fatty acids to confirm the identification of isopropyl esters. The corresponding isopropyl esters eluted from the chromatographic column almost a minute earlier than the *n*-propyl esters under the conditions we used and give distinctly different mass spectra. The *n*-propyl esters give stronger molecular ions and strong M-41 ions. The isopropyl esters give weaker molecular ions, and in addition to the M-41 ions give very strong M-42 ions. These differences alone enable one to recognize them readily [15].

In general we have found that ants living in a tropical environment have higher melting substances in their Dufour glands than those that live in colder climates. All linear hydrocarbons from hexadecane (m.p. 18 °C) upwards are solids at ambient temperatures in temperate climates. The liquid nature of the secretion of *P. ferruginea* and *P.* sp. A are easily explained by the rich mixture of isopropyl esters and large proportion of liquid pentadecane. Even that of *P. flavicornis* will be a liquid in a tropical environment, the major component, heptadecane (m.p. 22.5 °C) will be a liquid and its melting point will be lowered by the small amounts of pentadecane, heptadecane and isopropyl palmitate (m.p. 13 °C) dissolved in it.

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