Cell biology / Biologie cellulaire

Formation and structure of food bodies in *Cordia nodosa* (Boraginaceae)

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Abstract

Cordia nodosa Lamark (Boraginaceae) is a myrmecophyte (i.e., plants housing ants in hollow structures) that provisions associated ants with food bodies (FBs) produced 24 h a day. Distributed over all the young parts of the plants, they induce ants to forage continually and so to protect the plants. Metabolites are stored in the inner cells of *C. nodosa* FBs as they form. In addition the peripheral cells have an extrafloral nectary-like function and secrete a substance that covers the FBs. The amalgam of these two functions, distinct in other known cases, is discussed taking into account the origin of FBs and extrafloral nectaries. *To cite this article: P.-J. Solano et al., C. R. Biologies 328 (2005).*

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Résumé

Formation et structure des corps nourriciers chez *Cordia nodosa* (Boraginaceae). *Cordia nodosa* Lamark (Boraginaceae) est un myrmécophyte (c'est-à-dire une plante abritant des fourmis dans des structures creuses) qui produit en permanence des corps nourriciers sur toutes les parties jeunes de la plante, incitant les fourmis à fourrager et donc à protéger ces parties. Lors de la formation des corps nourriciers, il y a mise en réserve de substances dans les cellules profondes, plus une fonction de type nectaire extrafloral où les cellules périphériques sécrètent un produit qui s'étale à l'extérieur. L'amalgame de ces deux fonctions, habituellement séparées, est discuté en se référant à l'origine commune des corps nourriciers et des nectaires extrafloraux. *Pour citer cet article : P.-J. Solano et al., C. R. Biologies 328 (2005).*

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1. Introduction

Ant–plant interactions are very common and this relationship can vary from a facultative non-specific 'diffuse' relationship to an obligatory specific and symbiotic association. In diffuse relationships, ants forage for prey on the foliage of plants that attract them with food rewards such as extra-floral nectar (EFN) and/or food bodies (FBs), and in turn are protected against defoliating insects [1,2]. In myrmecophytes, or plants housing ants in hollow structures called domatia, the relationship is strict and necessary to the survival of both partners. In exchange for housing and sometimes EFN and/or FBs, the ants protect their host myrmecophytes from herbivores, competing plants, encroaching vines and fungal pathogens, or supply them with nutrients [2–4].

As EFN is mainly composed of sugary substances, FBs play an essential role in myrmecophyte–ant symbioses as they often contain relatively large amounts of proteins in addition to lipids and/or carbohydrates [1–3,5–7]. Also described on several non-myrmecophytic plants, the importance of FBs was noted in myrmecophytic *Acacia, Cecropia, Macaranga* and *Piper* [5–10].

O'Dowd [5], who reported that the elements stored in the FBs' inner cells occupy most of the cytoplasm, classified FBs according to their development and anatomy. They can derive from (1) epidermal tissue and can be single (*Piper*) or multi-celled, or from (2) sub-epidermal tissue (*Cecropia, Macaranga*), and they possibly bear an epidermal cell layer (*Cecropia*).

We examine here the structure of the FBs produced by the Amazonian myrmecophyte *Cordia nodosa* Lamark (Boraginaceae) [3], and how best to categorize them using O'Dowd's classification scheme [5].

2. Materials and methods

Samplings were conducted in the French Guiana primary rainforest around the field station *Les Nouragues* (4°5′N, 52°41′W), where the density of *C. nodosa* individuals varies from 3 to 20 per hectare, with *Allomerus octoarticulatus* Mayr being the most frequent associated ant species [11,12]. We looked for FBs on 50 *C. nodosa* individuals 1.5–2.4 m in height (the largest individuals can exceptionally reach 9–10 m)

bearing fruit and noted the behaviour of patrolling ants.

After zones of FB production were located, we collected samples of *C. nodosa* foliage and inflorescences in order to conduct histological studies. Samples for light microscopy were fixed with FAA (formalin, acetic acid, and alcohol), embedded in paraffin and tissue sections were tinted with toluidine blue contrasted with sodium molybdate. The samples for transmission electron microscopy (TME) were fixed with glutaraldehyde and osmic acid, and embedded with Epon. Contrasts were made using uranyl acetate and lead citrate [9].

3. Results

3.1. Food body distribution and production

We noted the presence of FBs in very different places on the plant: ovoid FBs (up to 0.2 mm in length; Fig. 1.1) were found near the veins on the abaxial face of the leaves, and spheroid FBs were found along young stems, the floral axis of the inflorescences and on immature fruits. Their production, although continuous, was very irregular and thus difficult to quantify. Both *Allomerus* and *Azteca* workers gathered them whole as is known for all other FBs (i.e. the presence of the extrafloral nectar-like secretion does not engender a particular behaviour).

3.2. Food body development and morphology

FB development occurs in a substomatal location (Fig. 1.2). The first stage consists of periclinal divisions in the subepidermic cell layer (Figs. 1.2 and 1.3). While growing, the first cells of the FB fill the substomatal gap and continued cell divisions cause an upward extension of the epidermis, which results in surface bumps (Fig. 1.3). Anticlinal divisions occur when the dividing cell layers emerge. Different division rates between the top and the base of the FB produces the basal neck and the 50-cell-plus FB takes a sub-spherical form (Figs. 1.1 and 1.4). Inner and peripheral cells do not mature in the same way. Inner cells enlarge, take a polyhedral form and have an active nucleus. The cells become flatter and more crenate (Figs. 1.4 and 1.5) towards the peripheral layer that is
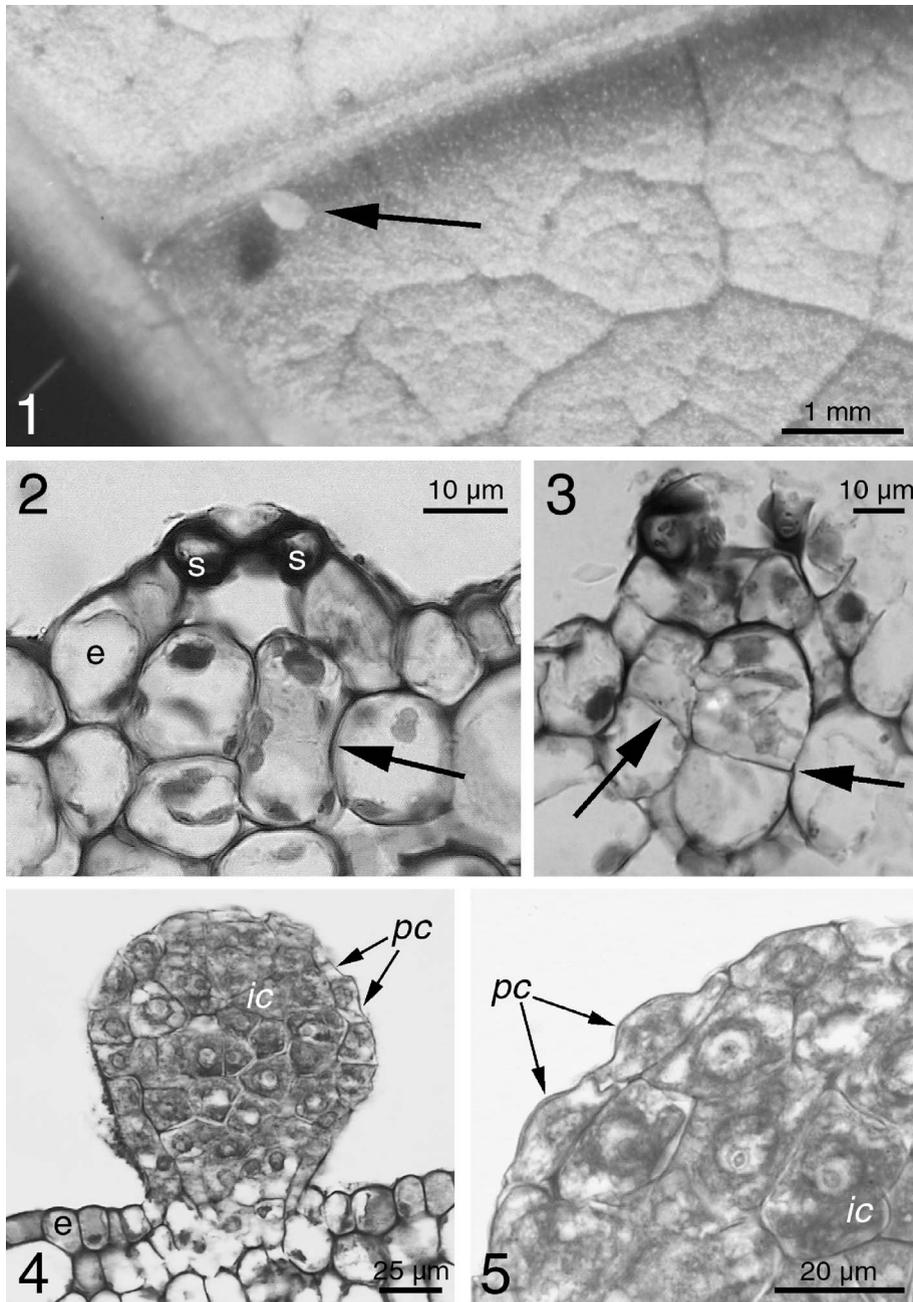
Fig. 1. (**1**) A food body (arrow) on a *Cordia nodosa* abaxial leaf face. The form is more ovoid than those developing on the stems or the fruits. (**2**) Light microscopy of the first stage of the emergence of an FB on the stem of *C. nodosa*. Sub-epidermal cells elongate, beginning to divide (arrow) under a stomata (s: guard cells of the stomata; e: epidermal cell layer). (**3**) Synthesis of the cell wall between two sub-epidermal cells at the end of division (arrows). The FB begins to emerge. (**4**) Fully emerged FB on *C. nodosa* from a young stem. Inner cells (*ic*) can be distinguished from peripheral cells (*pc*) flatter and in continuity with the epidermis (e). (**5**) Close-up of the peripheral cells (*pc*) and the inner cells (*ic*) of an FB of *C. nodosa*. Note the polyhedral form of the inner cells and the flat form of the peripheral cells.
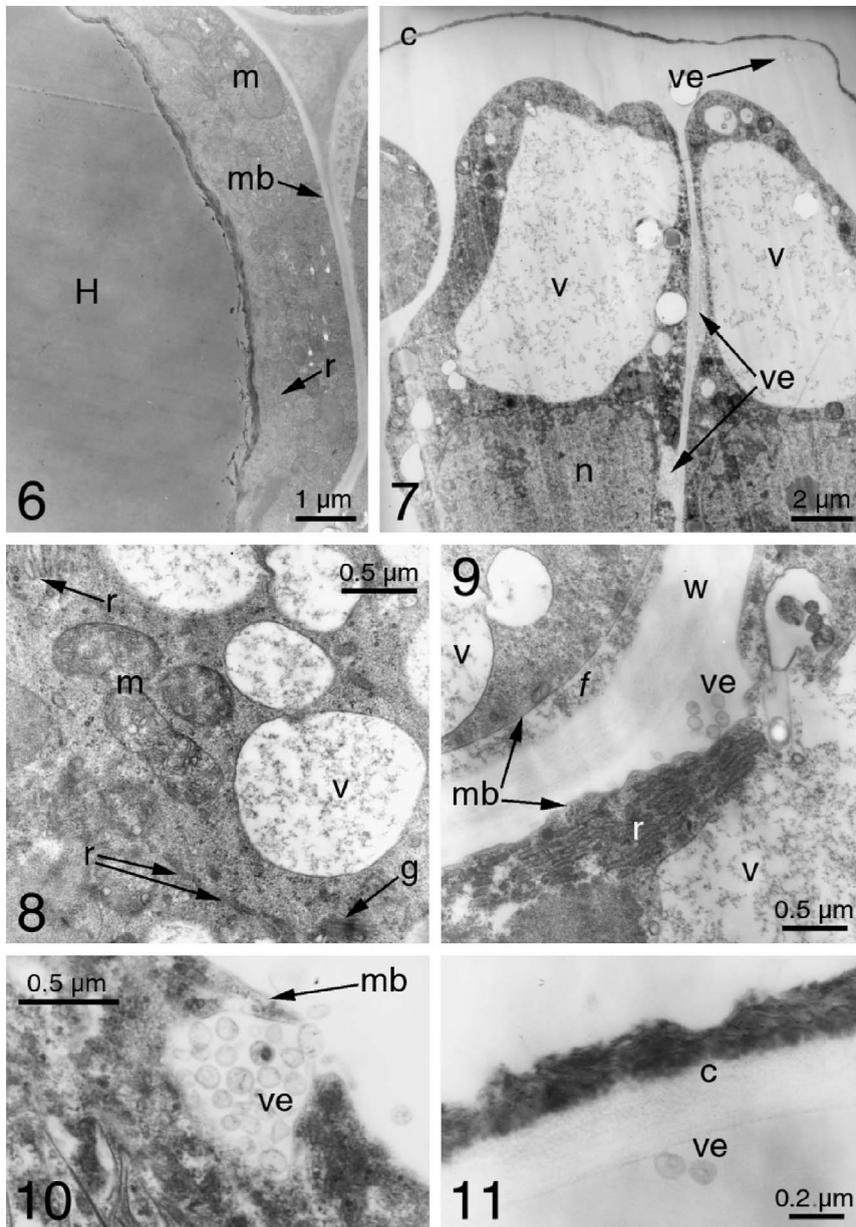
Fig. 1 (*continued*). (**6**) Ultrastructure of an inner cell of a *Cordia nodosa* FB. An important vacuole with homogeneous content (H) occupies the major part of the cell volume. The cytoplasm is rejected onto the edge of the cells, where mitochondria (m) and the endoplasmic reticulum (r) can be seen (mb: plasmalemma). (**7**) Ultrastructure of two peripheral cells of a *C. nodosa* FB. A vacuole with fleecy content (V) occupies a large volume. The nucleus (n) is lobed and occupies a large part of the cell. Vesicles (ve) are numerous in the apoplasm, between the cells and under the surface of the FB, covered by a dark substance (c). (**8**) Ultrastructure of 'fleecy' vacuoles (V) of a peripheral cell of *C. nodosa* FB. Their form implies that they are probably organized in a 3D reticulate system. Note the mitochondria (m), the endoplasmic reticulum (r) and the Golgi apparatus (g). (**9**) Close-up of the apoplasm between two peripheral cells of *C. nodosa* FB. On both sides of the cell wall (W) secretions through the plasmalemma (mb) accumulate in two ways: by exocytosis *via* groups of vesicles (ve), probably coming from the activity of the close endoplasmic reticulum (r), or through a fleecy-looking substance (f) similar to the content of the vacuole (V). (**10**) Close-up of the exocytosis of a group of vesicles (ve). (**11**) Two vesicles (ve) are close to reaching the surface of the *C. nodosa* FB. They are covered by a dark substance (c) that is probably an accumulation of the secretions.

continuous and can be considered as an epidermis. The guard cells, never observed on the apical zone of the FBs, probably necrotise during FB development. Neither vascular tissue nor transfer cells have been found between the FBs and the underlying vascular tissues.

3.3. Food body ultrastructure

The TEM observation of *C. nodosa* FBs confirms the differences seen by light microscopy between inner and peripheral cells. The inner cells are characterized by vacuoles occupying most of the cell volume and showing an important mass in the cytoplasm, whose content appears homogeneous (Fig. 1.6). Cytoplasmic activity is reflected in the numerous mitochondria and a lobed nucleus.

The peripheral cells can have globular vacuoles forming a single volume (Fig. 1.7), or a system of numerous vesicles with a reticulate 3D form that could derive from the edges of the endoplasmic reticulum's cisternae (Fig. 1.8). Their content is always light and fleecy. There is a high level of synthesis because the cytoplasm of the peripheral cells have numerous mitochondria, dictyosomes, and a well-developed endoplasmic reticulum (Figs. 1.8 and 1.9). Secretory activity is illustrated through small vesicles (less than 0.1 μm) that form outside the cells, whose content originates in the vacuoles (Figs. 1.7, 1.8 and 1.10). They are expelled in groups and can evolve in two ways. Some of them migrate through the apoplasm to the surface of the FB (Fig. 1.11). Others seem to burst between the membrane and the cell wall and pour their fleecy content into the apoplasm. The released substances can passively spread to the surface of the FBs, whose cuticle has two distinct layers (Fig. 1.11). The inner layer, the cuticle itself, is light and homogeneous, while the dark, heterogeneous and irregular external layer probably represents the dried accumulation of the secreted substance.

4. Discussion

FBs are produced on all the young parts of *C. nodosa* individuals, including the inflorescences, as described for *Ampelopsis* [5] and some *Macaranga* species [8]. This differs therefore from that of myrmecophytes, whose FBs are produced in specific zones or organs, such as the trichilia, situated at the base of the leaf petioles of *Cecropia*, with FB production limited to the afternoon [9,10], the stipules or sheathing leaf bases of *Piper* [13], the leaflets of *Acacia* [6], or both the leaves and stipules in other *Macaranga* species [8]. Because FB production is unpredictable, limited neither to a particular part of the plant nor time of day, the plant induces its associated ants to continually patrol its foliage, inflorescences and even immature fruits.

Unlike for *Cecropia peltata* or *C. obtusa*, the original guard cells do not remain on the top of *C. nodosa* FBs [9,10] nor do the stomata remain in most FBs [5]. Most of the cytoplasm of the inner cells of *C. nodosa* FBs is occupied by stored elements similar to those of *Acacia* or *Macaranga* that contain lipids [2, 6,8], but further analysis will determine which types of metabolites are stored in these inner cells. All the stages in the secretory activity of the peripheral cells are similar to typical nectar synthesis, progression and secretion in the peripheral cells of extrafloral nectaries (EFNs): a pre-nectar is temporarily accumulated in the vacuoles; then, during nectar secretion, the endoplasmic reticulum is very abundant, vesicles form then fuse with the plasmalemma and discharge the nectar outside the secretory cells by exocytosis [14], where it accumulates and becomes available for ants. The similarity between *C. nodosa* FBs (i.e., numerous vesicles; single-volume vacuoles absent) and EFN vesicles is great as vacuoles are not prominent in nectar-secreting cells [15]. As a result, peripheral cells likely secrete sugary substances, but the secretion of lipids cannot be excluded as this occurs frequently in secretory trichomes. Due to the cellular structure of FBs some proteins are also present, but they cannot satisfy the needs of the ant colonies. In this case the plant fuels ant foraging activity through its energy-rich FBs, and receives biotic protection [16], mostly through the predatory behaviour of associated ants. *A. octoarticulatus*, the ant most frequently associated with *C. nodosa*, is a remarkable group hunting species [12,17]; however, contrary to what was first thought, *A. octoarticulatus* colonies do not provide their host *C. nodosa* with nutrients [4].

By their anatomy and development, *C. nodosa* FBs belong to the second type defined by O'Dowd [5]: of sub-epidermal origin, they are multicelled, have a continuous external cell layer, a sub-spherical form and a pedicel. Nevertheless, *C. nodosa* FBs differ from all

other FBs. Their structure, noted for the first time to our knowledge, permits two types of activity: the intracellular storage of metabolites in inner cells, a typical FB role, and a nectary-like secretion by peripheral cells. EFNs are considered as more archaic structures than FBs, so that in the genus *Macaranga* a 'graded myrmecophily' was characterized by the relative presence of EFNs and FBs among the species [8,18]. The origins of EFNs and FBs are similar. For example, patches of glandular trichomes in Leguminosae could be primitive homologues of more organized EFNs [19, 20], while FBs originate in the trichomes [5,10].

In conclusion, *C. nodosa* belongs therefore to the restricted group of myrmecophytes that, in addition to housing ants, furnishes them with food in the form of FBs. Their dual production, likely sugary substances secreted by peripheral cells and lipids stored by inner cells, could indicate that these FBs have conserved an archaic character from secretory trichomes while acquiring inner storage cells, or that in general FBs and EFNs are not only two complementary structures, but perhaps also related. This production induces workers of associate ant species to forage on all parts of the host plant and fuels them with energetic food, while they hunt for the prey to satisfy their nitrogen requirements. Consequently, they protect their host plant, particularly the young, most vulnerable parts where FBs are produced.

Acknowledgements

References

[1] S. Koptur, Extrafloral nectary-mediated interactions between insects and plants, in: E.A. Bernays (Ed.), Insect–Plant Interactions, CRC Press, Boca Raton, FL, USA, 1992, pp. 81–129.

[2] M. Heil, D. McKey, Protective ant–plant interactions as model systems in ecological and evolutionary research, Annu. Rev. Ecol. Syst. Evol. 34 (2003) 425–553.

[3] D.W. Davidson, D. McKey, The evolutionary ecology of symbiotic ant–plant relationships, J. Hymenopt. Res. 2 (1993) 13–83.

[4] P.J. Solano, A. Dejean, Ant-fed plants: comparison between three geophytic myrmecophytes, Biol. J. Linn. Soc. 83 (2004) 433–439.

[5] D.J. O'Dowd, Pearl bodies as ant food: an ecological role of some leaf emergences of tropical plants, Biotropica 14 (1982) 40–49.

[6] F.R. Rickson, The ultrastructure of *Acacia cornigera* L. Beltian body tissue, Am. J. Bot. 62 (1975) 913–922.

[7] R.C. Fisher, A. Richter, W. Wanek, V. Mayer, Plants feed ants: food bodies of myrmecophylous *Piper* and their significance for the interaction with *Pheidole bicornis* ants, Oecologia 133 (2002) 186–192.

[8] B. Fiala, U. Maschwitz, Food bodies and their significance for obligate ant-association in the tree genus *Macaranga* (Euphorbiaceae), Bot. J. Linn. Soc. 110 (1992) 61–75.

[9] M. Belin-Depoux, P.-J. Solano, C. Lubrano, J.-R. Robin, P. Chouteau, M.-C. Touzet, La fonction myrmécophile de *Cecropia obtusa* Trecul (Cecropiaceae) en Guyane française, Acta Bot. Gal. 144 (1997) 289–313.

[10] F.R. Rickson, Ultrastructural differentiation of the Müllerian body glycogen plastid of *Cecropia peltata* L., Am. J. Bot. 63 (1976) 1272–1279.

[11] P.J. Solano, S. Durou, B. Corbara, A. Quilichini, P. Cerdan, M. Belin-Depoux, J.H.C. Delabie, A. Dejean, Myrmecophytes of the understory of French Guianian rainforests: their distribution and their associated ants, Sociobiology 41 (2003) 605–614.

[12] A. Dejean, A. Quilichini, J.H.C. Delabie, J. Orivel, B. Corbara, M. Gibernau, Influence of its associated ant species on the life history of the myrmecophyte *Cordia nodosa* in French Guiana, J. Trop. Ecol. 20 (2004) 701–704.

[13] F.R. Rickson, S.J. Risch, Anatomical and ultrastructural aspects of the ant-food cell of *Piper cenolacum* C. DC (Piperaceae), Am. J. Bot. 71 (1984) 1268–1274.

[14] A. Fahn, Secretory tissues in vascular plants, New Phytol. 108 (1988) 229–257.

[15] E. Echeverría, Update on intracellular transport. Vesiclemediated solute transport between the vacuole and the plasma membrane, Plant Physiol. 123 (2000) 1217–1226.

[16] M.E. Frederickson, Ant species confer different partner benefits on two neotropical myrmecophytes, Oecologia 143 (2005) 387–395.

[17] A. Dejean, P.-J. Solano, J. Ayroles, B. Corbara, J. Orivel, Arboreal ants build a trap to ambush and capture prey, Nature 434 (2005) 973.

[18] S.J. Davies, S.K.Y. Lum, R. Chan, L.K. Wang, Evolution of myrmecophytism in Western Malesian *Macaranga* (Euphorbiaceae), Evolution 55 (2001) 1542–1559.

[19] R. Schnell, G. Cusset, M. Quenum, Contribution à l'étude des glandes extra-florales chez quelques groupes de plantes tropicales, Rev. Gén. Bot. 70 (1963) 269–342.

[20] L. Pascal, E.F. Motte-Florac, D. McKey, Secretory structures on the leaf rachis of Caesalpinieae and Mimosoideae (Leguminosae): implications for the evolution of nectary glands, Am. J. Bot. 87 (2000) 327–338.