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10. The methanol-soluble portion of the residue from urine after extraction with ether and lyophilization to dryness was treated with diazomethane and then subjected to gas-liquid chromatography (GLC) analysis on a 3.8 percent OV-101 or 3.0 percent OV-17 column at 150° to 250°C with quantitation of the methylated mercapturic acids using a flame ionization detector. The structural identity of each excreted mercapturic acid was verified by methylation and GLC-mass spectroscopy (chemical ionization) comparison with authentic standards.
11. The standard plant root detoxification assay involved addition of [*S*-alkyl-^{14}C]EPTC sulfoxide (1 nmole) or [*N*-alkyl-^{14}C]butylate sulfoxide (0.5 nmole) to the 17,300*g* supernatant of root homogenates (2.5 to 320 mg of fresh tissue weight equivalent) and GSH (10 μmole) in phosphate buffer (0.1*M*, *p*H 6.8, 1.1 ml). After 2 hours incubation at 25°C, the unmetabolized [^{14}C]EPTC sulfoxide or [^{14}C]butylate sulfoxide was recovered by extraction with chloroform and subjected to liquid scintillation assay. Enzyme activity is expressed as picomoles of thiocarbamate sulfoxide cleaved per milligram of enzyme (fresh tissue weight equivalent) per hour.
12. In a separate but similar study in which were assayed the GSH *S*-transferase activity with EPTC sulfoxide at a high substrate level (1 μmole), corn root enzyme (5 to 40 mg of fresh tissue weight equivalent) prepared with insoluble polyvinylpyrrolidone (*4*) and 40 minutes of incubation, the same relationship was noted of increasing enzyme activity with increasing R-25788 level. In this case, the enzyme activity was elevated two- to fourfold at high antidote levels as compared with no antidote treatment.
13. *S*-(*N*,*N*-[^{14}C]Diisobutylcarbamyl)-GSH formed as a metabolite of [^{14}C]butylate sulfoxide (*11*) was identified by cochromatography with an authentic unlabeled standard (*6*) on two-dimensional TLC with 0.5-mm silica gel F_{254} chromatoplates developed in the first direction with pyridine, isopropanol, water, glacial acetic acid system (100 : 20 : 20 : 1) and in the second direction with the same system in the ratio of 50 : 55 : 45 : 2.
14. Supported in part by PHS grant PO1 ES00049 and PHS fellowship 1 F22 ES00681 (the latter to J.P.H.). R. L. Holmstead of this laboratory assisted in the GLC—mass spectroscopy studies. We thank R. A. Gray and F. M. Pallos of Stauffer Chemical Co. for samples of [^{14}C]EPTC, [^{14}C]-butylate, R-25788, and R-29148. The corn and oat seeds were provided by C. W. Crum of Dekalb Agricultural Research, Inc., DeKalb, Ill., and C. O. Qualset of the University of California, Davis, respectively.

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Nectar: Its Production and Functions in Trumpet Creeper

Abstract. *Studies of the trumpet creeper,* Campsis radicans *(L.) Seem. (Bignoniaceae), reveal five distinct nectary systems, a phenomenon never before reported among temperate zone plants. Ant activity, centered around the four extrafloral systems, clearly demonstrates the ant-guard symbiosis usually associated only with tropical or subtropical species. Floral nectar, an attractant for hummingbird and bumblebee pollinators, differs chemically from the ant-attracting nectar produced extraflorally.*

The production of nectar by plants and the attraction of certain insects to nectar have been of long-standing fascination to scientists (*1*). Much work has been channeled into the study of floral nectaries and the pollinators associated with them (*2*). Investigations of extrafloral nectaries have been limited until recently. These structures are located on the outer floral (*3*) and vegetative parts, including petioles (*4*), sheaths (*5*), and leaf margins (*6*). A result of this recent work has been a broadening of the field of animal-plant interactions. In recent years many ant-plant associations have been recognized in the tropics and subtropics, ranging from casual temporary alliances to mutualistic symbioses in which the participants are dependent on each other for survival. An example of the latter is the bull's horn acacia, *Acacia cornigera* L., and the acacia ant, *Pseudomyrmex ferruginea* F. Smith (*7*). The ants inhabit the enlarged hollowed stipular thorns and feed on a balanced diet of nectar from petiolar nectaries and Beltian bodies, modified leaf tips rich in protein. In addition to removing encroaching plants, the resident ants drive off invading insects by biting and stinging.

The trumpet creeper, *Campsis radicans*, one of the few temperate representatives of the tropical and subtropical family Bignoniaceae, is a common woody vine of the eastern and midwestern United States. Throughout its range it is host to several genera of ants. The relationship is not obligatory, although varying degrees of protection may be offered by different ant species in return for the extrafloral nectar produced by the plant. Trumpet creeper is possibly unique among temperate species, and certainly among a small number of all nectariferous plants, in possessing five nectary systems. The four extrafloral systems, located on the petiole (Fig. 1, a, b, and d), calyx (Fig. 1, e and f), corolla, and fruit (Fig. 1, h and i), are each visited regularly by ants. This report is perhaps the first documented case of nectaries occurring on developing fruits of any species. The ovarian (floral) nectary (Fig. 1g) aids in attracting the primary and secondary pollinators, hummingbirds and bumblebees, respectively. The extrafloral systems are also present on the Old World *Campsis grandiflora* as well as its hybrid *Campsis* × *tagliabuana* (*C. radicans* × *C. grandiflora*).

The aggressive climbing habit of the trumpet creeper has made it a familiar sight along hedgerows and fences of the Midwest. The flowers are five-parted and bilaterally symmetrical, each with a short tubular calyx and a flaring, tubular, showy, bright orange corolla, borne in dense terminal corymbs of 12 to 35 flowers (n = 21.6) (Fig. 1, c and j). Leaves are opposite, pinnately compound, each with 7 to 11 leaflets. The hybrid closely resembles its American parent in gross morphological features, the most conspicuous differences being the looser inflorescences and the larger flowers. Flowering, which occurs in both taxa from early July until early September, follows the "cornucopia" pattern as described by Gentry (*8*). Fruiting continues into late September, averaging 2.6 fruits per inflorescence.

Material was studied during the summers of 1972 and 1974 from native populations in southern Illinois, and naturalized populations were examined in southeastern New York. The hybrid was grown and observed in the nursery of the Cary Arboretum. Living material was fixed and stored in FAA (formalin, acetic acid, and ethyl alcohol), dehydrated in a tertiary butyl alcohol series, and embedded in TissuePrep. Serial sections were made at 10 μm, stained with safranin, and counterstained with fast green or with Delafield's hematoxylin. In order to determine the carbohydrate components, we collected nectar at different times during the day throughout the growing season, using microcapillary spotting tubes. Analysis was accomplished by thin-layer chromatography (*9*).

The individual extrafloral nectaries are minute and may be easily overlooked by the casual observer. The nectaries are generally circular in outline with a well-defined structure of a cup cavity with a base, surrounded by a wall or rim. Data concerning location, number, and size of the different nectaries and nectar composition are given in Table 1.

The petiolar nectaries are the first to secrete. The nectaries on the youngest three or four pairs of petioles on each branch

Table 1. Nectary systems of the trumpet creeper. Abbreviations: S, sucrose; G, glucose; F, fructose.

Location	Average number	Average height (mm)	Average diameter (mm)	Ratio of sugars		
				S	G	F
Extrafloral						
Petiole—adaxial surface	15.6/petiole	0.14	0.27	1	1	1
Calyx—abaxial lobes	20/flower	0.17	0.26	3	2	1
Corolla—abaxial lobes	25/flower	0.14	0.23	3	2	1
Fruit—scattered	200/fruit	0.15	0.19	1	1	1
Floral						
Ovarian—base of style	1/flower	1.73	3.98	0	1	1

were found to be active at a given time. The individual nectaries, each lying in a slight depression of the petiole, are clustered irregularly on the adaxial surface from the node to an average distance of 10.5 mm.

The second nectary system, located on the outer calyx, begins secreting nectar when the flower buds are only 0.5 to 1 mm long and continues through flowering. The exterior of the corolla near the bases of the lobes is the site of the third nectary system. It becomes evident as the corolla tube elongates, when the overlapping lobes unfold. Secretion begins about 1 day prior to flowering and continues for the life of the flower, an average of 4 days.

The ovarian nectary is a massive annular ring that produces copious nectar in the short period just prior to and during flowering. The relatively soft, young fruits have nectaries randomly scattered on the surface. These cupular nectaries begin secretion soon after the corolla falls away, while the developing fruit undergoes rapid elongation. Secretion ceases as the wall of the fruit becomes leathery. At full maturity the walls of the fruits are woody.

Extrafloral nectar of the trumpet creeper is very effective as an attractant for ants. The four extrafloral nectary systems of nursery-grown plants of the hybrid *Campsis* × *tagliabuana* attracted *Formica* sp. (*fusca* group) and *Lasius alienus americanus* (*10*). Illinois populations of *Campsis radicans* attracted ants of the genera *Formica* and *Crematogaster*. Observations of ant activity and aggression in Illinois populations revealed differences in the degree of protection according to the species of ant present.

One Illinois population (eight large plants) was inhabited exclusively by the large ant *Formica* sp. (*fusca* group). These ants did not flee when disturbed and were loath to abandon their positions to capillary tubes. No evidence of flower robbing or fruit foraging was noted on these plants. The ants were effective in protecting the young leaves, flowers, and fruits.

In a second population (14 large plants) ants of the species *Crematogaster lineolata* were observed feeding from all four of the extrafloral nectary systems as they became functional. These relatively small ants displayed no aggression when foreign elements were introduced onto the flowers, petioles, and fruits. In fact, most fled. A significant number (7 percent) of the corollas had holes cut into the sides of the tubes, typical of nectar-robbing bumblebees.

The complex systems of nectar production and release would be of little value were it not for the highly evolved social organization of ants. An ant colony relies on anatomical and behavioral specializations of its members for the performance of diverse tasks required to maintain the group. The development of a plant protection function by ants is strengthened when examined in light of the alarm-defense systems (*11*) proven to be in operation in ant groups. Imminent danger triggers the release of pheromones by individuals, which sets off a defense response. Once the recognition of danger to the host plant is established, the same defense pattern may apply. The degree of plant protection can be correlated directly with the state of advancement of the ant species' alarm-defense system. Wilson and Regnier consider the panic-alarm response, an unorganized retreat from danger, to be a primitive response. The aggressive "stand and hold" behavior, or the deliberate movement toward the disturbance and possible attack of intruders, is considered advanced.

As a result of our observations and from anatomical studies of *Campsis*, we conclude that the four extrafloral nectary systems serve as ant-attracting systems, resulting in a symbiotic relationship. The level of plant benefit from the relationship is dependent on the species of ant present. The evidence extends the range of the ant-guard phenomenon, formerly associated only with the tropics and subtropics, to temperate regions.

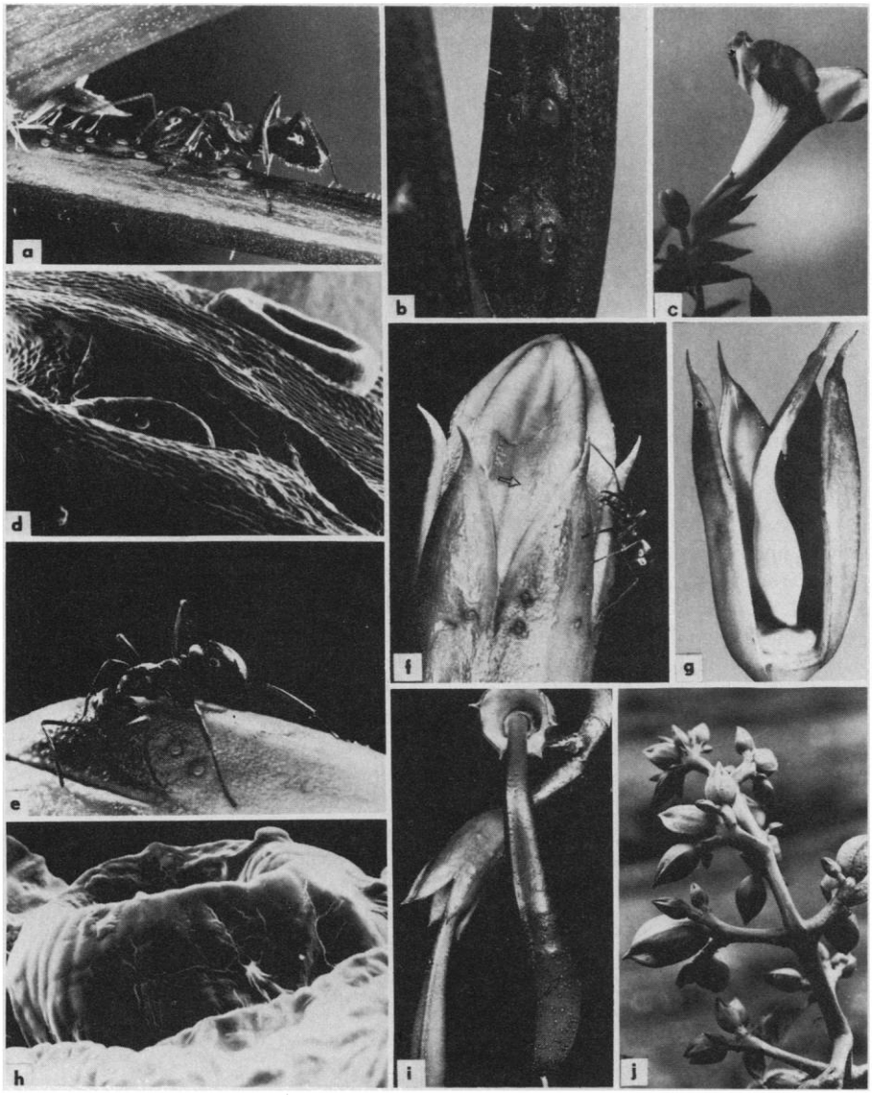

Fig. 1. (a) Lower part of petiole with *Formica* sp. feeding from nectaries (× 3⅓). (b) Adaxial surface of petiole showing nectaries and nectar droplets (× 4⅔). (c) Longitudinal view of flower (× ⅓). (d) Scanning electron micrograph of two petiolar nectaries (× 500). (e) Ventral surface of flower bud showing nectaries near base of calyx lobe and *Formica* sp. (× 3⅓). (f) Flower bud with calyx expanded and emerging corolla tube, showing calyx and corolla nectaries (arrow) with *Formica* sp. (× 2⅔). (g) Flower with corolla and part of calyx removed to show annular nectary at base of ovary (× 2). (h) Scanning electron micrograph of nectary on surface of fruit (× 1000). (i) Immature fruits with tiny scattered nectaries (× ⅓). (j) Inflorescence of *Campsis* × *tagliabuana* (× ⅓).

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Nuclear Protein Matrix: Association with Newly Synthesized DNA

Abstract. *The residual structural framework of the cell nucleus, termed the nuclear protein matrix, is associated with newly synthesized DNA in regenerating rat liver. One minute after rats are injected with [^{3}H]thymidine, more than 90 percent of the total tritium in nuclear DNA is associated with the matrix DNA although this DNA comprises only 25 percent of the total nuclear DNA. In contrast, the bulk DNA, 75 percent of total nuclear DNA, contains less than 8 percent of the total labeled DNA. The percentage of total labeled DNA associated with the bulk DNA increases for 30 minutes after injection and decreases correspondingly in the matrix DNA.*

We have reported the isolation and characterization of a new structural protein matrix in mammalian nuclei (*1*). Isolated rat liver nuclei contain a residual framework structure, which maintains the spherical shape of the nucleus. When isolated, this nuclear structure extends from residual components of the nuclear envelope to the nuclear interior where it forms a distinctive internal matrix that connects to a residual nucleolar structure.

The isolated residual framework structure termed the nuclear protein matrix is essentially free of the DNA, RNA, and phospholipids of the nucleus; it accounts for approximately 12 percent of the total nuclear proteins and is composed primarily of three distinct nonhistone acidic polypeptide fractions of 60,000 to 70,000 daltons. Other physical and chemical fractionation procedures indicate that the nuclear protein matrix consists predominantly of nuclear acidic proteins and is essentially free of histone proteins. In addition, amino acid analyses of the total matrix protein fraction demonstrate a ratio of acidic to basic amino acids of 1.46.

In accord with these findings, previous investigators have observed complex residual structures in nuclei from which the chromatin had been extracted. Zbarsky and his colleagues detected residual structural components in the nucleus after treatment with 2*M* NaCl solutions (*2*). Busch and his co-workers were the first to observe a ribonucleoprotein network extending throughout the nucleus, from the nuclear membrane to the nucleolus (*3*). Busch and Smetana suggested that these nuclear elements might be involved in RNA processing and transport (*4*). The networks observed by these previous investigators were complex nuclear structures enclosed by an intact nuclear envelope. The nuclear protein matrix which we have isolated may be a residual protein component of the above structures.

We now report on the possible function for the nuclear protein matrix related to DNA synthesis. During the isolation of the protein matrix, distinct fractions of DNA are removed with each sequential extraction. Approximately 75 percent of the total nuclear DNA can be removed by reducing the magnesium chloride concentration from 5.0 m*M* to 0.2 m*M* in the nuclear suspension medium. We have termed this DNA, bulk DNA.

Electron microscopic studies reveal that the DNA remaining with the extracted nuclei (approximately 25 percent of the total nuclear DNA) is associated with the nuclear protein matrix. This total matrix DNA can be further fractionated by 2.0*M* NaCl into a soluble DNA component and a residual matrix DNA, which remains tightly associated with the nuclear protein matrix.

We have observed the temporal sequence of labeling of the above DNA fractions after a single injection of [^{3}H]thymidine into the hepatic portal vein of regenerating rat livers. Results with this regenerating rat liver system indicate that the newly synthesized DNA is associated with the matrix DNA.

Liver nuclei were isolated from male rats (Sprague-Dawley, 250 to 300 g, Charles River) (*5*). Livers were excised quickly, minced with a scalpel and homogenized at 0°C with ten strokes at 1300 rev/min in a Potter-Elvehjem apparatus with two volumes of sucrose-TM buffer (0.25*M* sucrose, 50 m*M* tris, *p*H 7.4, 5 m*M* $MgCl_2$ at 22°C). After filtration through four layers of cheesecloth, the homogenate was centrifuged at 780*g* for 10 minutes to yield a crude nuclear pellet. The pellet was resuspended in 2.2*M* sucrose TM buffer and centrifuged at 40,000*g* for 90 minutes. This purified nuclear pellet was washed twice in 0.25*M* sucrose TM buffer and subjected to the following sequence of treatments to obtain the isolated nuclear protein matrix.

Table 1. Distribution of [^{3}H]thymidine label, as a function of time after injection in nuclear DNA fractions from regenerating rat liver. Each value represents the mean ± the standard error of the mean, for three separate experiments at 20, 24, and 30 hours after partial hepatectomy. For each experiment, livers from four separate animals were pooled and DNA and radioactivity determinations were performed in triplicate. The specific activities are shown in Fig. 1A.

Time after injection (min)	Percent of total DNA	Percent of total label	Ratios	
			Specific activity to total nuclear DNA specific activity	Specific activity to bulk DNA specific activity
		Bulk DNA		
1	74.8 ± 3.6	7.6 ± 2.3	0.05 ± 0.01	1.0
5	72.0 ± 1.2	19.4 ± 2.4	0.19 ± 0.02	1.0
10	74.3 ± 2.3	35.6 ± 4.6	0.36 ± 0.05	1.0
30	75.0 ± 1.1	67.2 ± 3.1	0.78 ± 0.05	1.0
		Total matrix DNA		
1	25.2 ± 3.6	92.4 ± 2.3	3.50 ± 1.1	46.7 ± 14.8
5	28.0 ± 1.2	80.6 ± 2.4	2.06 ± 0.32	11.4 ± 2.6
10	25.7 ± 2.3	64.4 ± 4.5	1.97 ± 0.46	5.37 ± 0.58
30	25.0 ± 1.1	32.8 ± 3.1	1.24 ± 0.09	1.49 ± 0.23
		High-salt (2.0M NaCl) soluble matrix DNA		
1	23.6 ± 3.4	82.2 ± 3.3	3.73 ± 0.72	44.7 ± 14.4
5	26.5 ± 1.7	74.6 ± 2.3	2.41 ± 0.16	11.1 ± 2.4
10	23.2 ± 2.8	58.2 ± 4.9	1.99 ± 0.45	5.42 ± 0.58
30	23.7 ± 0.9	31.6 ± 3.2	1.25 ± 0.11	1.51 ± 0.22
		Residual matrix DNA		
1	1.7 ± 0.3	9.8 ± 1.3	5.6 ± 1.9	77.6 ± 2.8
5	1.5 ± 0.4	6.0 ± 0.6	2.6 ± 0.8	14.9 ± 5.3
10	2.6 ± 0.5	6.2 ± 1.7	2.0 ± 0.7	5.61 ± 1.5
30	1.2 ± 0.3	1.2 ± 0.7	1.0 ± 0.3	0.97 ± 0.2