Planta (1998) 205: 305–318

Planta

Nectar-carbohydrate production and composition vary in relation to nectary anatomy and location within individual flowers of several species of Brassicaceae

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Received: 21 July 1997 / Accepted: 19 September 1997

Abstract. Nectar-carbohydrate production and composition were investigated by high-performance liquid chromatography and enzymology in nine species from five tribes of the Brassicaceae. In six species (*Arabidopsis thaliana* (L.) Heynh., *Brassica napus* L., *B. rapa* L., *Lobularia maritima* (L.) Desv., *Raphanus sativus* L., *Sinapis arvensis* L.) that produced nectar from both lateral nectaries (associated with the short stamens) and median nectaries (outside the long stamens), on average 95% of the total nectar carbohydrate was collected from the lateral ones. Nectar from these glands possessed a higher glucose/fructose ratio (usually 1.0–1.2) than that from the median nectaries (0.2–0.9) within the same flower. Comparatively little sucrose was detected in any nectar samples except from *Matthiola bicornus* (Sibth. et Sm.) DC., which possessed lateral nectaries only and produced a sucrose-dominant exudate. The anatomy of the nectarial tissue in nectar-secreting flowers of six species, *Hesperis matronalis* L., *L. maritima*, *M. bicornus*, *R. sativus*, *S. arvensis*, and *Sisymbrium loeselii* L., was studied by light and scanning-electron microscopy. Phloem alone supplied the nectaries. However, in accordance with their overall nectar-carbohydrate production, the lateral glands received relatively rich quantities of phloem that penetrated far into the glandular tissue, whereas median glands were supplied with phloem that often barely innervated them. All nectarial tissue possessed modified stomata (with the exception of the median glands of *S. loeselii*, which did not produce nectar); further evidence was gathered to indicate that these structures do not regulate nectar flow by guard-cell movements. The numbers of modified stomata per gland showed no relation to nectar-carbohydrate production. Taken together, the data on nectar biochemistry and nectary anatomy indicate the existence of two distinct nectary types in those Brassicacean species that possess both lateral and median nectaries, regardless of whether nectarial tissue is united around the entire receptacle or not. It is proposed that the term "nectarium" be used to represent collectively the multiple nectaries that can be found in individual flowers.

Key words: Brassicaceae – Carbohydrate (floral nectar) – Floral nectary (anatomy) – Nectary (intrafloral location) – Nectary phloem – Stoma (modified)

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Introduction

Nectaries are structures that produce and secrete nectar, a carbohydrate-rich solution, to the plant's surface. In flowers, these glands may perform a vital function in sexual plant reproduction by attracting biotic agents of pollination. Nectar composition is important as it relates to pollinator type. Based on the three carbohydrates predominating in most nectars, sucrose/(glucose + fructose) ratios have permitted designations of nectar type (sucrose-dominant and -rich, hexose-dominant and -rich) found to be favoured by specific nectarivores (Baker and Baker 1983). For example, floral nectar in most species of the Brassicaceae is dominated by hexose and readily collected by butterflies and short-tongued bees (Percival 1961; Baker and Baker 1983).

Carbohydrate composition of nectar also affects the stability of the liquid honey elaborated from it. Low glucose/water ratios in honey are important in delaying its crystalization; the high glucose content in floral nectar of the Brassicaceae (Percival 1961) can expedite honey granulation (Shuel 1989; Kevan et al. 1991).

In the Brassicaceae, the possession of multiple outgrowths of nectarial tissue per flower has been known at least since 1848 (Caspary, cited in Davis 1992). These outgrowths emerge from the receptacle and their differences in morphology, location and size among species within the family have received interest from plant taxonomists (see Deng and Hu 1995; Davis et al. 1996).

Within individual flowers of certain non-Brassicacean taxa, nectaries also can occur more than once. Here,

however, at least some of these multiple nectaries are adnate to perianth or reproductive parts. Daumann (1931) described multiple locations for nectaries within flowers of the dicotyledons *Aristolochia* sp., *Limnanthemum* sp. and *Mimulus cardinalis*, and Kunze (1995) found two nectary locations in flowers of the asclepiads *Matelea argentinensis* and *M. reticulata*. The monocotyledons *Epidendrum O'Brienanum*, *Iris* sp., *Sagittaria chinensis* and *S. sagittifolia* also possess multiple nectaries per flower (Daumann 1931), and two separate nectary types in each flower of *Sternbergia clusiana* attract different insect visitors (Dafni and Werker 1982).

Determinations of nectar composition from multiple nectaries within single flowers apparently are lacking, except for the preliminary detection of a disparity in nectar-carbohydrate composition from separate nectary locations inside individual flowers of *Arabidopsis thaliana* (Davis 1992). Here we have extended the comparison of nectar-carbohydrate profiles from intrafloral nectary locations, to eight additional Brassicacean species representing four more tribes from the subfamilies Siliquosae and Siliculosae. In addition, for six of these species, for which mostly descriptions or line drawings only have been published, we investigated the anatomy of the floral nectaries and related specific structural features of the nectaries to the disparity in nectar-carbohydrate production from different gland locations within single flowers. The anatomy of the floral nectaries of the three other species is described elsewhere: *Brassica napus* L. and *B. rapa* L. (see Deng and Hu 1995; Davis et al. 1996) and *Arabidopsis thaliana* (L.) Heynh. (Norris 1941; Davis 1992, 1994).

Materials and methods

Plant material. In total, nine species were maintained as follows. In June-July 1995, four plants each of the weeds *Sinapis arvensis* L. and *Sisymbrium loeselii* L. were transplanted from various locations on the University of Saskatchewan campus (52.07 N, 106.38 W) and grown outdoors in pots. Open flowers were removed and then inflorescences bagged with nylon mesh (approx. 2/mm) to prevent visits by large insects. Periodically, bags were removed and unvisited open flowers immediately excised for nectar collection (see below) in the laboratory nearby. The four plants of *Hesperis matronalis* L. grew nearby in a garden plot and were treated similarly. Plants were watered regularly without splashing the bags.

The remaining six species studied (at least three plants each) were grown from seed in growth chambers at 20 °C. These included three ecotypes of *Arabidopsis thaliana* (L.) Heynh. (Nottingham Arabidopsis Stock Centre, University Park, UK), rapid-cycling lines of *Brassica napus* L. and *B. rapa* L. (Crucifer Genetics Cooperative, Madison, Wis., USA) and *Lobularia maritima* (L.) Desv., *Matthiola bicornus* (Sibth. et Sm.) DC. and *Raphanus sativus* L. (Thomson & Morgan, Ipswich, UK). All seed was sown on Sunshine Mix (Fisons Horticulture Inc., Mississauga, Ontario, Canada) except *A. thaliana* (Redi-Earth; WR Grace & Co., Ajax, Ontario, Canada). Plants received a photoperiod of 16 h (200 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$)/8 h darkness, except the initial cohort of *A. thaliana* ecotype Columbia, which experienced continuous illumination (150 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$). Bagging of flowers was unnecessary in the growth chambers.

Anatomy of floral nectaries. Nectaries from open, nectar-bearing flowers were examined by light and scanning-electron microscopy (SEM). For light microscopy, flowers were fixed in FAA or 2% glutaraldehyde (GA) in 25 mM $NaPO_4$ buffer (pH 6.8), then dehydrated (*n*-butanol or ethanol) and embedded in Paraplast wax (Oxford Labware, St. Louis, Mo., USA) or LR White plastic resin (London Resin Co., Basingstoke, Hampshire, UK), respectively. Wax sections (10 μm) were cut on a Leitz-Wetzlar rotary microtome and heat-fixed to slides before staining with Toluidine blue O. Sections were dewaxed in xylene and mounted permanently under coverslips in Permount. Plastic-embedded material was sectioned (1 μm) and similarly stained before mounting in immersion oil under coverslips. Sections were photographed with Kodak TMax 100 film using a Zeiss Universal microscope.

For SEM, flower bases fixed overnight in GA were rinsed in $NaPO_4$ buffer, post-fixed in 1% OsO_4 in buffer, and then dehydrated in a graded acetone series. Tissues were critical-point-dried with liquid CO_2 (Polaron Instruments, Watford, UK), mounted on aluminum stubs using double-sided tape, gold-coated with an Edwards Sputter Coater S150B and examined at 30 kV with a Philips 505 scanning-electron microscope. Photographs were taken with Polaroid 665 positive/negative film.

Laboratory culture of intact flowers. To enhance the volume of floral nectar available for collection and analysis, intact mature buds of *Arabidopsis* and *Lobularia* (both normally yielding little nectar) and *Sisymbrium* were cultured in the laboratory (Davis 1992). Immediately after excision from the plant, cut pedicels of buds nearing anthesis were inserted through pin-holes in Parafilm (American Can Co., Greenwich, Conn., USA; Fig. 1A) that covered a 25-mL vial of 10% (w/v) sucrose (reagent-grade, Sigma) solution, pH 4.8. To minimize the post-secretion evaporation of water from nectar (Corbet et al. 1979), bud-laden vials were placed into 5 mL distilled water held inside a 150-mL beaker and sealed therein with three sheets of Parafilm (to maintain high humidity) before wrapping in aluminum foil and incubating for 24 h at 22 °C.

Nectar collection and analysis. Normally 9–12 flowers per plant were sampled for nectar, except *A. thaliana* (1–4 per plant) (Table 1). Under a dissecting microscope, wicks cut from Whatman No.1 filter paper (McKenna and Thomson 1988) were used to soak up all available nectar from the floral nectaries (Fig. 1B, 2A). For *Hesperis* and *Matthiola*, the large lateral sepals (Fig. 3A,G) first had to be retracted to allow access to the nectar. To assist collection from non-cultured species possessing viscous nectar, wick tips first were dabbed in deionized water. Often more than one wick was required per nectar droplet. For species possessing droplets at both lateral and median nectaries in single flowers, nectar was collected on separate wicks. All wicks were stored dry in labelled envelopes at room temperature until analysis.

Two different techniques were utilized to quantify major nectar carbohydrates. Enzymatic analysis (Davis 1997a) was conducted only for the initial cohort of *A. thaliana* ecotype Columbia. All other samples were analysed by high-performance liquid chromatography (HPLC) as follows. Wicks were soaked in 0.5–1.0 mL of pure distilled water in disposable centrifuge tubes (Eppendorf) on ice and shaken intermittently. After sample solutions were pressed through a sterile syringe filter (pore size 0.20 μm; Corning Glassworks, Corning, N.Y., USA), 100 μL per sample was analysed by a Waters (Waters Chromatography, Milford, Mass., USA) 625 metal free gradient HPLC apparatus equipped with a Waters 712 Wisp autosampler. Carbohydrates (glucose, fructose and sucrose) were separated using a Carbo Pac PA1 pellicular anion-exchange column (250 mm long, 4 mm i.d) coupled with a Carbo Pac PA1 guard column (150 mm long, 4 mm i.d.; both from Dionex, Sunnyvale, Calif., USA). Elution of the carbohydrates was carried out with a mobile phase of 80 mM NaOH at a flow rate of 1.0 mL $\cdot$ min^{-1}. The carbohydrates were detected by a pulsed amperometric detector (Waters Model 464) with a dual gold electrode and triple pulsed amperometry at a sensitivity of 50 μA. The electrode was maintained at the following potentials and durations: $E_1 = 0.05$ V ($T_1 = 0.299$ s); $E_2 = 0.60$ V ($T_2 = 0.299$ s); $E_3 = -0.80$ V ($T_3 = 0.499$ s). Carbohydrates eluting

from the column were plotted by a Maxima 820 chromatography work station.

Standards (0–50 mg · l^{-1}) containing each of the three major nectar carbohydrates were run in duplicate and routinely gave strong linear relationships ($r = 0.99$) of concentration to peak area. Quantities of glucose, fructose and sucrose in nectar samples were calculated from these linear equations, means calculated from the duplicate samples, and appropriate corrections made for wick dilution. Although other carbohydrates can exist in nectar in minor amounts (Percival 1961; Baker and Baker 1983), no oligosaccharides were detected in nectar of *B. rapa* (*B. campestris*) (Low et al. 1988). As there were no other simple sugars detected during the 15-min analyses, total nectar carbohydrate production per flower was estimated by the summation of these three carbohydrates. To deduce total nectar carbohydrate per flower in species bearing both lateral and median nectaries, quantities were summed; the percentage released from each nectary type was calculated.

Results

Nectary anatomy

Tribe Alysseae – Lobularia maritima. These flowers were unusual by their possession of eight separate nectaries, among the highest number of glands per flower in the Brassicaceae (Hildebrand 1879). All ten flowers examined bore four pairs of nectaries, two lateral and two median (Fig. 1C,D), in accordance with Caspary (cited in Hildebrand 1879) and Schulz (1936). Each short stamen was flanked on either side by a lateral nectary (Fig. 1C–E) that received a relatively rich supply of phloem alone (Fig. 1G) and possessed 5.50 ± 0.25 (SE, range 3–8, $n = 32$ lateral nectaries from nine flowers of the same plant) modified stomata (Fig. 1F,G) on its uppermost surface.

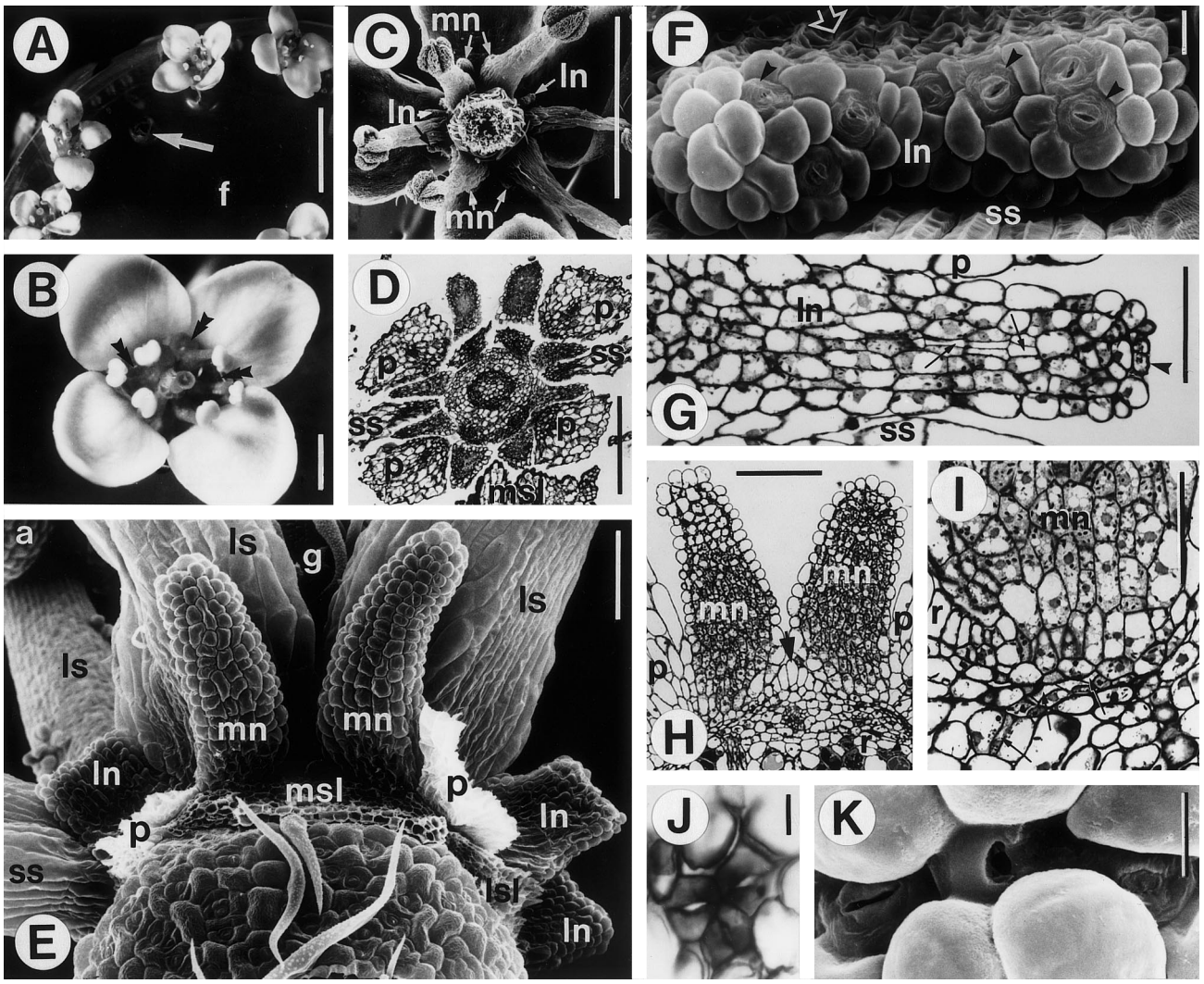
Bayer (1905) reported the median nectaries as absent in *Alyssum maritimum* Lam. (synonym for *L. maritima*). Here, however, median glands were present and each was filiform and situated close to the other nectary of the pair (Fig. 1E,H), at the insertion of each petal and external to the filament of each long stamen (Fig. 1D,E, H). Paired median nectaries were not confluent at their bases, being separated by larger vacuolate cells of the median-sepal base (Fig. 1H) and then smaller vacuolate cells of the receptacle (Fig. 1I left). Similarly, SEM examination of 13 long-stamen/petal interfaces from 6 flowers, and serial sectioning of another flower, confirmed that the median and lateral nectaries also were isolated and not connected between the stamen filament and petal claw. Median nectaries received a small supply of phloem, and only to the base (Fig. 1I). On their tips and sometimes facing the developing silicule, 0.73 ± 0.22 (range 0–4, $n = 33$ median nectaries) modified stomata per nectary were detectable, partially concealed by the adjacent, overarching epidermal cells (Fig. 1J,K). Multiple stomata were usually adjacent (Fig. 1J,K).

Tribe Brassiceae – Sinapis arvensis. Four nectaries per flower were detected (Fig. 2D) as reported earlier (Jordan 1886; Bayer 1905). Surrounding each of the two lateral nectaries were the bases of the gynoecium, short stamen, and two long stamens and petals (Fig. 2D, E), such that nectaries indeed appeared as compressed or truncated prisms (Bayer 1905; Schulz 1936). In a total of six flowers examined, we did not find any bilobed lateral nectaries (Schulz 1936), although such variation in *Brassica* spp. is common (Davis et al. 1996). The nectary surface possessed 18.0 ± 1.4 (range 10–23, $n = 8$ nectaries from four flowers of the same plant) modified stomata found only on the upper surface; modified stomata were almost exclusively solitary and mature (Fig. 2D). Numerous strands of phloem (without xylem), often consisting of three or four sieve elements grouped per strand, were found within each lateral gland (Fig. 2E).

Outside the bases of each pair of long stamens, each median nectary projected as an outgrowth (Fig. 2D,F) described as ovoid or tongue-like (Bayer 1905; von Hayek 1911; Clemente Munoz and Hernandez Bermejo 1978). The surface of each median nectary bore 43.5 ± 1.7 (range 37–51) modified stomata, usually mature and solitary (Fig. 2D). Phloem alone entered the base of each median nectary, but only as a few small strands and barely into the gland interior (Fig. 2F,G). A narrow gap between petals and adjacent long stamens allowed a thin nectarial connection between median and lateral nectaries in 19% of such regions (not shown).

Tribe Brassiceae – Raphanus sativus. Four nectaries per flower were encountered (Fig. 2B), as reported previously (Bayer 1905; Schulz 1936; Norris 1941; Clemente Munoz and Hernandez Bermejo 1978). Each of the two lateral nectaries was delimited by the gynoecium, two petals, and one short and two long stamens (Fig. 2C). von Hayek (1911) related that the face of the lateral nectary was "konkave"; within and bordering this central horizontal depression occurred modified stomata (Fig. 2C) that were mostly still immature, usually solitary and numbered 64.8 ± 2.9 (range 50–78, $n = 8$ nectaries from four flowers of the same plant) per nectary. As in *Sinapis* (Fig. 2E), the gland interior possessed numerous strands of phloem; no tracheary elements of xylem accompanied the sieve elements, verifying the statements and drawings of Arber (1931) and Norris (1941).

Bases of the long stamens did not completely meet the petal claws in 75% of such junctions from five flowers, allowing narrow bands of nectarial tissue to connect lateral nectaries to adjacent median ones (not shown). Each of the two median nectaries per flower was situated external to and between each pair of long stamens (Fig. 2B) and, like *Sinapis* (Fig. 2F,G), was supplied relatively poorly with phloem (and see Deng and Hu 1995, Fig. 19). Here glands were usually peg- or fan-shaped (Davis et al. 1996), not cylindrical or elongate (Bayer 1905; Villani 1905; Schulz 1936; Clemente Munoz and Hernandez Bermejo 1978). Median nectaries bore 27.4 ± 2.5 (range 15–40) modified stomata each. Neither Arber (1931) nor Norris (1941) labelled median nectaries in their drawn transections, perhaps confirming that these glands sometimes are aborted (von Hayek 1911). Of 27 flowers studied here, 2 lacked a median



Figs. 1–3. Abbreviations: *a*, anther; *g*, gynoecium; *ln*, lateral nectary; *ls*, long stamen; *lsl*, lateral sepal; *mn*, median nectary; *msl*, median sepal; *p*, petal; *pd*, pedicel; *r*, receptacle; *ss*, short stamen

Fig. 1A–K. *Lobularia maritima*. **A** Flowers each with pedicel inserted through hole (*arrow*) in Parafilm (*f*) covering vial of sucrose solution. **B** Glistening nectar droplets (*double arrowheads*) within cultured flower. **C** Flower with petals removed to reveal seven of its eight nectaries at the stamen bases. **D** Sectioned flower showing relatively small cells of the eight nectaries. **E** Flower base after removal of perianth, showing five of the nectaries. **F** Modified stomata (*arrowheads*) atop lateral nectary, bruised at its base (*arrow*) by adjacent petal. **G** Phloem (*arrows*) and modified stoma (*arrowhead*) on lateral nectary sectioned longitudinally. **H** Median nectaries sectioned longitudinally showing separation by innermost cells of median sepal (*arrow*). **I** Sieve tube of elements (*arrows*) reaching median nectary base. **J** Median nectary tip showing three adjacent modified stomata. **K** Linear triplet of modified stomata on median gland tip. Bars = 5 mm (**A**), 1 mm (**B**,**C**), 250 μm (**D**), 100 μm (**E**,**H**), 50 μm (**G**,**I**), 10 μm (**F**,**J**,**K**)

gland on 1 side only; the remainder possessed 2 median nectaries.

Tribe Sisymbrieae – Sisymbrium loeselii. Nectarial tissue was continuous around the receptacle (Fig. 2H), the lateral nectaries completely enclosing each filament of the short stamens in the pentagonal arrangement (Fig. 2I) described by Velenovsky (1883) and Bayer (1905). In the median plane, the glandular tissue extended from each outermost apex of the lateral nectaries as swollen bands or ridges (von Hayek 1911; Schulz 1936) separating the long stamens from the petals (Fig. 2H,I), thus completing the flower's "Drüsenring" (Schweidler 1911) accurately drawn by Velenovsky (1883). In four flowers from two plants, half the median nectaries were bilobed slightly (Fig. 2H right, L), the other half smooth (Fig. 2H left): each type could occur together (Fig. 2H). Lobing of the median glands may account for Knuth's (1908) report of two, four or six nectaries per flower. Never were the median glands found so prominent as shown in Deng and Hu (1995).

Phloem alone supplied the glandular ring. Most prevalent were the strands of sieve elements that were directed toward and entered the lateral nectarial tissue on either side of the short stamen (Fig. 2J,K). Moreover, sieve tubes were detected in the regions both above and below the insertion of the short stamen (not shown). Strands of phloem also extended into the median

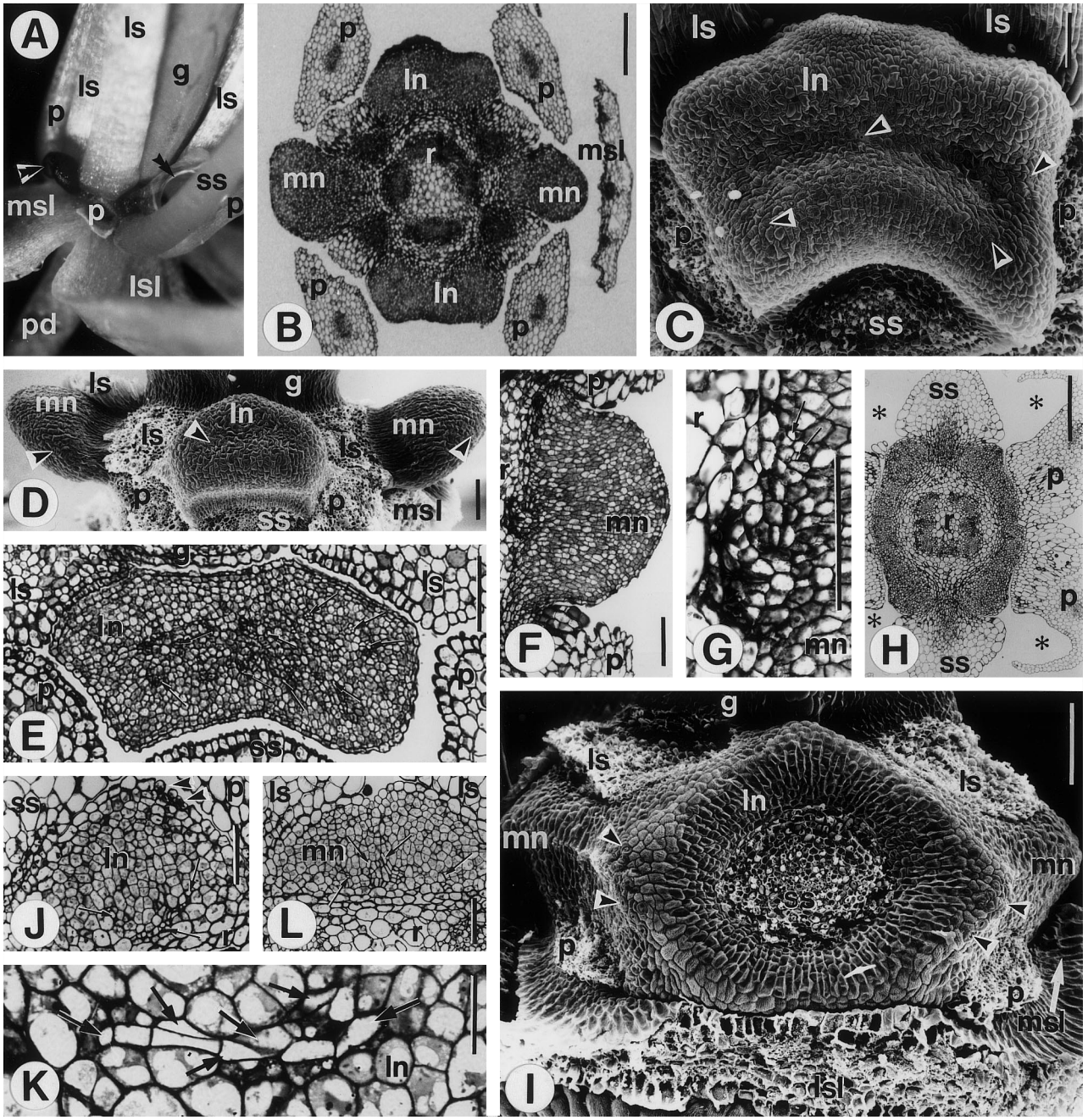


Fig. 2A–L. *Brassica napus* (**A**); *Raphanus sativus* (**B**,**C**); *Sinapis arvensis* (**D–G**); *Sisymbrium loeselii* (**H–L**). **A** One petal removed to reveal two nectar droplets (*double arrowheads*), a small one (*left*) on the apex of the median nectary and a large one (*right*) on the lateral nectary, partially hidden behind the short stamen. **B** Transverse section through the four nectaries, separated by incoming long stamens. **C** Modified stomata (*arrowheads*) along central horizontal depression of lateral nectary. **D** Modified stomata (*arrowheads*) on median and lateral nectaries. **E** Oblique section through lateral nectary, *arrows* indicating some of the many bundles of sieve elements. **F** Absence of phloem inside median nectary sectioned longitudinally. **G** Base of nectary shown in **F**, showing sieve elements (*arrows*) that only penetrate a few cells into the gland. **H** Transverse section showing insertion of short stamens that interrupt the annular nectarium. *Asterisks* denote depressions in petal claws where each nectar droplet accumulates. **I** Lateral aspect of flower, showing confluence between lateral and median nectaries; modified stomata (*arrowheads*) on two apices of lateral nectary. **J** Transverse flower section through one apex of the lateral nectary showing phloem (*arrows*) and modified stomata (*arrowheads*). **K** Tube of multiple sieve elements (*arrows*) within a lateral apex, veering toward nectary epidermis at upper right. **L** Phloem (*arrows*) entering bilobed median nectary just below insertion of long stamens. Bars = 250 μm (**B**, **H**), 100 μm (**C–G**, **I**), 50 μm (**J**, **L**), 20 μm (**K**)

nectarial tissue, even where it projected upward between the diverging petal claws (Fig. 2L), at a level just below insertion of the long stamens.

Not a single modified stoma was found on the 8 median nectaries examined; furthermore, no nectar droplet was ever observable on the petal claw opposite these sites in 49 flowers (including 42 cultured ones, from 4 plants) examined. Instead, the modified stomata were concentrated in four specific locations – the apices of the lateral-nectary pentagons that connected to the median

regions (Fig. 2I,J). Here, the modified stomata usually were clumped with others, only 15% being solitary, and their number averaged 32.4 ± 1.7 (range 25–42; $n = 12$ lateral apices from three flowers of the same plant) per lateral apex. The four nectar droplets per flower accumulated precisely here, opposite each lateral-nectary apex, through the gap between each petal and short-stamen filament (Fig. 2H,J). At their bases, the narrow petal claws become "wedge-shaped" (Schulz 1936) and the nectar collected as droplets in the recessions opposite these gaps (Fig. 2H).

Tribe Hesperideae – Hesperis matronalis. Flowers possessed two lateral nectaries but completely lacked median ones (Fig. 3B), verifying earlier descriptions (Velenovsky 1883; Norris 1941; Dvorák 1968). The junction of adjacent long stamens occurred immediately above the attachment point of the opposing sepal (Fig. 3B). Owing to this blockage (also see Dvorák 1968), the narrow ridges of nectarial tissue projecting backwards from the two lateral nectaries ("Ausläufer"; Bayer 1905) and skirting between each long stamen and petal (Fig. 3B,D) never coalesced in the median plane (nine flowers from two plants).

Each lateral nectary was confined between two petals and long stamens and surrounded the short stamen (Fig. 3B–D), 83% of the time in a completely annular fashion (Fig. 3B,D), a phenomenon reported in the larger survey of Dvorák (1968) as rare. The face (opposing the short stamen) of most nectaries was flat, but 11% of glands appeared bilobed, having a central crevice (Fig. 3D). However, two completely separated structures on either side of the short stamen (Schulz 1936, Fig. 130A), were never encountered. The glandular ring was considerably thicker above than below the short stamen, and at the latter often had small lobes (Fig. 3B right, D). When not annular, the portions of the lateral nectary flanking the short stamen (Fig. 3C) failed to meet below it, leaving the ring open. Modified stomata, which were almost always solitary (Fig. 3D, E), numbered 27.8 ± 1.6 (range 20–36; five flowers from same plant) per nectary. Interestingly, less than 30% were found on the nectary face, where nectar accumulated between the three stamens as a droplet (and see Knuth 1908); the remaining stomata occurred on the nectary sides facing the petals (Fig. 2B) and, less often, the long stamens.

Only phloem supplied the interior of each lateral nectary, corroborating the findings of Norris (1941). Sieve elements of variable dimensions (Fig. 3F) were detected in the glandular tissue both below the short stamen (not shown) and above it, the phloem residing only centrally as separate bundles (Fig. 3C). At that location, phloem entered the nectary base as particularly contorted sieve tubes (Fig. 3F).

Tribe Matthioleae – Matthiola bicornus. Like *H. matronalis*, median nectaries were absent in *M. bicornus* (Fig. 3H–J), confirming earlier observations (Schulz 1936; Dvorák 1967). However, four lateral nectaries extended as projections 0.5 mm long, one on each side of the short stamens (Fig. 3I,K). At their base and for part of their height, the nectaries were slightly concave around the filament of the short stamen (Fig. 3I,N). All modified stomata were confined to the distal half of the nectaries (Fig. 3K,M) and numbered 21.4 ± 1.0 (range 17–26; $n = 12$ nectaries from three flowers of the same plant) per gland. Modified stomata were mostly solitary and immature, and less commonly were located on the surface of the nectaries facing the gynoecium.

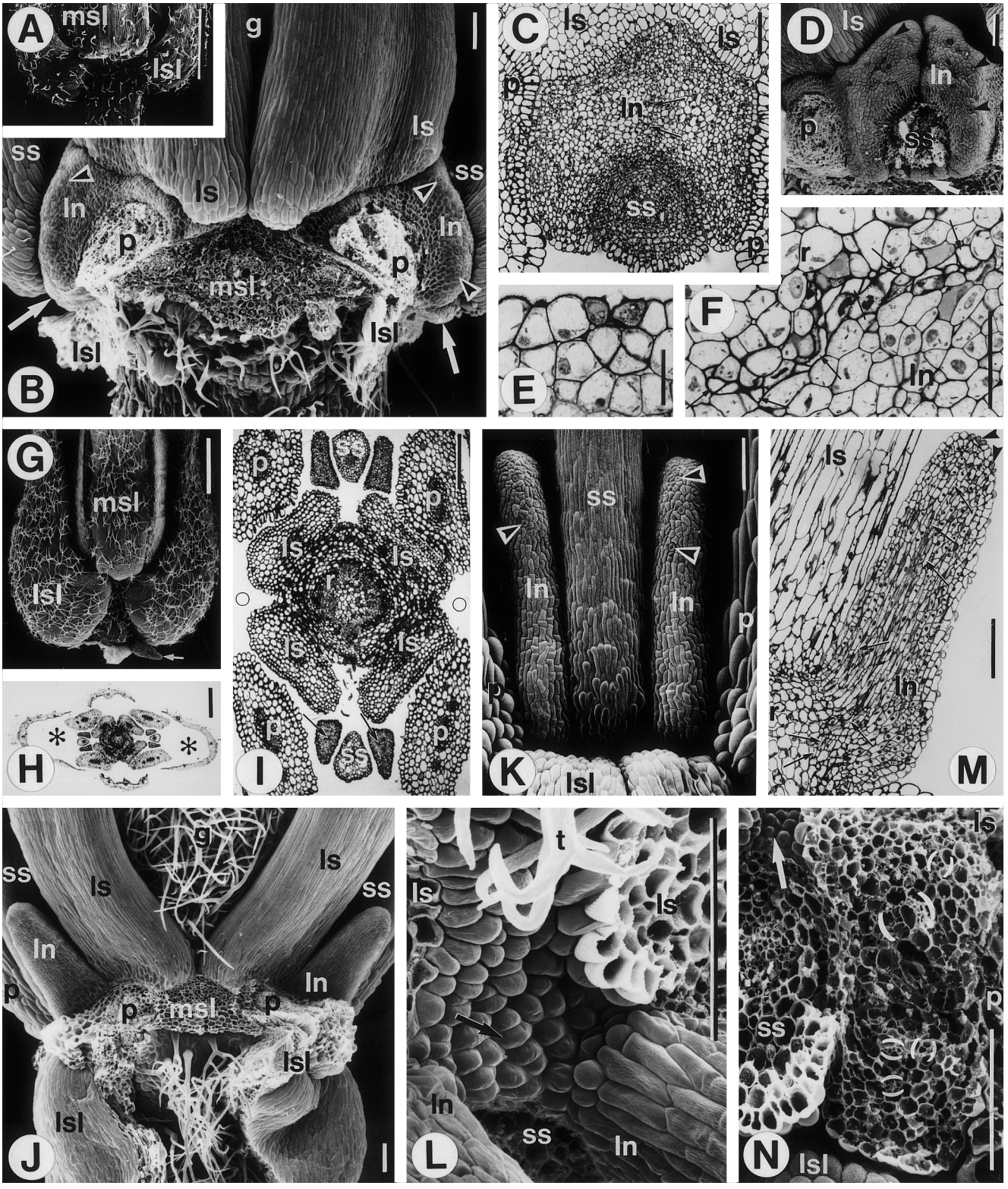
Petals, long stamens and lateral sepals were so closely fitting at their attachments that in six flowers, nectarial tissue was never observed to extend between then (Fig. 3J,N) unlike *H. matronalis* (Fig. 3B,D). Furthermore, in *M. bicornus* the bases of neighbouring nectaries (eight lateral positions available for examination in five flowers) did not connect below the short stamens (but see Schulz 1936 and Dvorák 1967), being kept separate by the larger cells of the sepals (not shown). However, above the same stamens, all nine lateral positions examined possessed small, isodiametric cells – often papillate on their outer epidermal wall (Fig. 3L,N top left) – that connected adjacent lateral nectaries as a short belt of variable size (Fig. 3L,N).

Phloem alone supplied the lateral glands (Fig. 3M). It consisted of sieve elements of variable diameter in four to six discrete bundles at the nectary base (Fig. 3N) that extended most of the nectary's height (Fig. 3I bottom), reaching approximately six cells below the modified stomata (Fig. 3M).

Nectar carbohydrate production per flower

Almost a 400-fold difference in average total nectar carbohydrate per intact (in situ) flower was detected between species (Table 1). With the exception of the moderately large flowers of *H. matronalis*, nectar-

Fig. 3A–N. *Hesperis matronalis* (**A–F**); *Matthiola bicornus* (**G–N**). **A** Saccate lateral sepals at flower base. **B** Perianth removed to show lateral nectaries completely encircling the short stamens (*arrows*) and extending between bases of petals and long stamens. Modified stomata are also shown (*arrowheads*). **C** Approximately longitudinal section through face of lateral nectary. Note phloem (*arrows*). **D** Bilobed nectary with extension between petal and long stamen (*left*). Modified stomata are indicated (*arrows*). **E** Modified stoma on nectary surface. **F** Sieve elements (*arrows*) of meandering sieve tube leading into nectary. **G** Base of flower showing saccate lateral sepals and squamule (*arrow*). **H** Transverse section showing nectar reservoirs (*asterisks*) formed by saccate lateral sepals. **I** Absence of nectarial tissue at median positions (*circles*). Phloem bundles (*arrows*) in lateral nectaries flanking short stamens. **J** Median view after removal of sepals and two petals. **K** Modified stomata (*arrowheads*) on lateral nectaries. **L** Junction of lateral nectaries above short stamen insertion, showing several cell layers of small, papillate epidermal cells (*arrow*) below larger cells intervening long stamens. *t*, gynoecial trichomes. **M** Sieve elements (*arrows*) and modified stomata (*arrowheads*) seen in radial longitudinal section through nectary. **N** Scanning-electron micrograph of interior of nectary base, showing bundles of sieve elements (*parentheses*) and small, papillate cells (*arrow*) between nectaries above short stamen. Note close proximity and interdigitation (*bottom right*) of adjacent floral parts. Bars = 1 mm (**A**, **G**), 0.5 mm (**H**), 250 μm (**I**), 100 μm (**B–D**, **J–N**), 50 μm (**F**), 20 μm (**E**)



carbohydrate production was related to flower size. Intraspecific comparisons indicate that significant differences in amount of nectar sugar per flower occurred between the haploid and higher levels of ploidy in *B. rapa* (Davis et al. 1994), and between the three ecotypes of *A. thaliana* which averaged 1.9-fold for in-situ flowers and 2.2-fold when cultured (Table 1). Furthermore, despite fewer than five plants being investigated per species (except *A. thaliana*), similar significant interplant differences in nectar-carbohydrate production per flower, averaging 2.3-fold, were observed (Table 2).

For most species investigated in vitro, flowers cultured individually on a sucrose solution had more than double the total nectar carbohydrate available in situ

Table 1. Proportion of total nectar carbohydrate produced in situ or in vitro at lateral-nectary locations (LN) within individual flowers of nine species from five tribes of the Brassicaceae. Tribe designations follow Schulz (1936). These are grand means (±SE) calculated from the averages per plant

Tribe	Species [ploidy]	Ecotype/ Variety	In situ			In vitro		
			No. plants (flowers)	Total nectar carbohydrate per flower (μg)	Total nectar carbohydrate from LN (%)	No. plants (flowers)	Total nectar carbohydrate per flower (μg)	Total nectar carbohydrate from LN (%)
Alysseae	*Lobularia maritima* (L.) Desv.	Carpet of Snow	1 (6)	4.52 ± 0.54	82.0[a] ± 6.8	4 (44)	23.2 ± 0.86	74.5[a] ± 2.6
Brassiceae	*Brassica napus* L.[b]	Rapid-cycling	4 (69)	386 ± 39.4	93.8 ± 3.4	–	–	–
	B. rapa L. [n][b]		3 (53)	54.8 ± 8.79	95.6 ± 0.60	–	–	–
	[2n][b]	Rapid-cycling	4 (71)	171 ± 19.6	92.9 ± 2.9	–	–	–
	[4n][b]	Rapid-cycling	4 (68)	194 ± 51.8	94.7 ± 0.89	–	–	–
	Raphanus sativus L.	Cherry Belle	3 (27)	230 ± 52.0	98.3 ± 0.46	–	–	–
	Sinapis arvensis L.		4 (35)	113 ± 25.9	92.3 ± 2.2	–	–	–
Hesperideae	*Hesperis matronalis* L.		4 (44)	41.2 ± 1.15	100	–	–	–
Matthioleae	*Matthiola bicornus* (Sibth. et Sm.) DC.	Night Scented	3 (37)	274 ± 54.4	100	–	–	–
Sisymbrieae	*Arabidopsis thaliana* (L.) Heynh.	Columbia	11 (11)	1.50 ± 0.17	100[a]	21 (29)	3.30 ± 0.25	97.4[a] ± 1.3
		Landsberg	4 (15)	2.06 ± 0.35	96.4[a] ± 1.9	18 (32)	5.79 ± 0.68	96.1[a] ± 1.1
		Wassilewskija	6 (7)	1.08 ± 0.20	100[a]	11 (29)	2.68 ± 0.28	100[a]
	Sisymbrium loeselii L.		1 (8)	95.8 ± 23.3	100[a]	4 (42)	121 ± 32.8	100[a]

[a]Within rows, mean totals of nectar carbohydrate from LN (%) are not significantly different (2-tailed *t*-tests, $P > 0.05$). [b]Data from Davis et al. (1994)

(Table 1). In *S. loeselii*, the 1.3-fold increase was also significant ($P < 0.04$).

Nectar carbohydrate production in relation to nectary location

Overall, lateral nectar was present in all 652 flowers examined from nine species, and 98.8% of those had nectar droplets at *both* lateral sides of each flower. Even in species possessing median nectaries, invariably it was the lateral ones that produced the bulk (average 94.6%; range 74.5–100%) of a flower's total nectar carbohydrate (Table 1).

Not only were median nectar droplets usually smaller than lateral ones (Fig. 1B, 2A), but median glands produced nectar less often. Of 568 flowers overall that had median nectaries, only 71.3% produced some median nectar. There were noteworthy differences among taxa; even though the Sisymbrieae were cultured as isolated flowers *ad libitum*, only 15.2% of them produced median nectar, compared to 97.9% in *L. maritima* (Alysseae) and 93.8% in tribe Brassiceae. As a result, median nectaries accounted for less than 10% of a flower's total nectar-carbohydrate production except in *L. maritima*, where they averaged 22% (Table 1).

Culturing tended to elevate the proportion of the total nectar carbohydrate available at the median nectaries (Table 1), although this increase was not statistically significant ($P > 0.05$) for any species. Moreover, culturing of flowers of *S. loeselii* failed to yield nectar (Table 1, Fig. 5N) at the nectarial tissue below the long stamens (Fig. 2H,L) or the relatively infrequent median nectaries (data not shown) in flowers of ecotype Wassilewskija of *A. thaliana* (Table 1, Fig. 5M). Of course, median nectar also was unavailable from *H. matronalis* and *M. bicornus* (Table 1, Fig. 5H,I) because those species lacked nectarial tissue in the median plane (Fig. 3B,I,J).

Nectar carbohydrate composition in relation to nectary location

In the six species that produced some median nectar, it possessed more fructose than glucose (Fig. 4C); glucose/fructose ratios ranged from 0.19 (Fig. 5D) to 0.92 (Fig. 5J). The average glucose/fructose ratio of 0.89 for median nectar of *S. arvensis* (Fig. 5G) was computed from data collected only from Plants 2–4, because the median nectar of Plant 1 was dramatically different, it averaging 5.7-fold more glucose than fructose (Fig. 4F).

In the lateral nectar of all species except *M. bicornus*, glucose usually exceeded fructose with ratios ranging from 0.91 (Fig. 5A) to 1.22 (Fig. 5E). Even in the few species or ecotypes where this ratio was below unity (Fig. 5A,M), in every plant having nectar available from both lateral and median nectaries, the glucose/fructose ratios always averaged higher in lateral nectar (Fig. 4B,C; Fig. 5A–G, J–L). The one exception was Plant 1 of *S. arvensis*, where median nectar had a much

Table 2. Range in average total nectar-carbohydrate production (μg) per flower (mean ± SE) between plants of the same species, and the ratio between high (H) and low (L) producing plants

Species [ploidy]	Treatment	No. plants	Range (L–H)	Ratio (H/L)
Brassica napus[a]	In situ	4	312–488[b]	1.56
B. rapa [n][a]	In situ	3	39.7–70.2[b]	1.77
[2n][a]	In situ	4	123–208[b]	1.69
[4n][a]	In situ	4	109–342[b]	3.15
Hesperis matronalis	In situ	4	38.0–43.5[c]	1.14
Lobularia maritima	In vitro	4	21.9–25.6[c]	1.17
Matthiola bicornus	In situ	4	150–413[b]	2.75
Raphanus sativus	In situ	3	175–334[b]	1.91
Sinapis arvensis	In situ	4	42.7–163[b]	3.82
Sisymbrium loeselii	In vitro	4	58.7–214[b]	3.64

[a]Data from Davis et al. (1994)
[b]Difference between H and L is statistically significant using 2-tailed *t*-tests, $P < 0.05$
[c]Difference between H and L is not significant

higher glucose/fructose ratio than the lateral nectar (Fig. 4E,F). In that plant, the median nectar differed dramatically from median nectar of all other species (cf. Fig. 4C,F). Excluding all 10 flowers of this unusual plant, on an individual flower basis, only 4.4% (14 of 319) of flowers overall produced nectar where the glucose/fructose ratio from their median nectaries exceeded that of their lateral nectaries.

The high content of sucrose and the predominance of fructose over glucose in lateral nectar of *M. bicornus* (Fig. 4G, 5I) set this species apart from all others studied. Its high sucrose/hexose ratio of 4.5 contrasted dramatically with that of other lateral nectar [range 0 (Fig. 5F) to 0.16 (Fig. 5L)] or median nectar [range 0 (Fig. 5F,G,J) to 0.28 (Fig. 5L)].

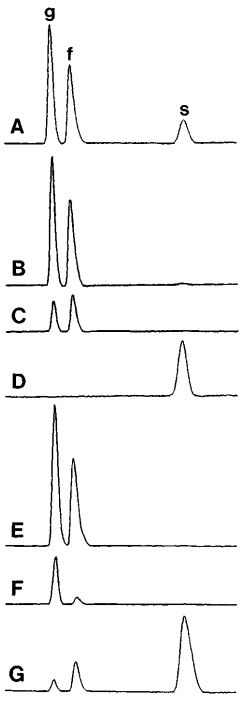


Fig. 4A–G. Representative HPLC profiles of carbohydrate standards and samples of nectar and culture medium. **A** Standard mixture of glucose (*g*), fructose (*f*), and sucrose (*s*); each sugar at a concentration of 25 mg · l^{-1}. **B** Nectar from lateral nectaries of cultured flower of *Lobularia maritima*. **C** Nectar from median nectaries of same flower as in **B**. **D** Culture medium immediately after nectar collection from same flower as in **B**. **E** Nectar from lateral nectaries of non-cultured flower of Plant 1 of *Sinapis arvensis*. **F** Nectar from median nectaries of same flower as in **E**. **G** Nectar from lateral nectaries of non-cultured flower of *Matthiola bicornus*

Whereas culturing raised the quantity of nectar sugar produced, nectar-carbohydrate composition remained essentially the same between in situ and cultured flowers of the same plant (Table 3). Regardless of whether nectar hexose composition was determined using HPLC (Fig. 5J) or enzymatic analysis (Fig. 5K), similar results were obtained for cultured flowers of ecotype Columbia of *A. thaliana*. Nectar from cultured buds (*A. thaliana*, *L. maritima*, *S. loeselii*) was composed predominantly of hexoses (Fig. 4B,C; Fig. 5A, J–N) that were absent in the culture medium (Fig. 4D), but in proportions not different from uncultured flowers (Table 3). These findings validate this experimental procedure of enhancing nectar yields for analytical purposes.

Discussion

Nectar production in relation to nectary anatomy. Every flower examined produced nectar at the lateral positions, whereas almost 30% of flowers possessing median nectaries lacked any nectar there. These findings corroborate the long-held knowledge that median nectaries in the Brassicaceae normally yield little, if any, nectar. Jordan (1886) referred to the median nectaries of *Arabidopsis thaliana* (as *Arabis thaliana*) as "ohne Verrichtung (steril)", and in *Brassica napus*, the lateral and median nectaries were considered "fertilen und sterilen" (Beutler 1930). However, advances in analytical techniques (HPLC and enzymology), the filter-paper wick method (McKenna and Thomson 1988) for gathering individual nectar droplets, and the culture of small flowers singly at high humidity thereby elevating nectar-sugar production and reducing nectar viscosity, all have contributed to allow the meagre quantities of nectar available at the median nectaries to be investigated.

Can nectary anatomy account for this disparity in nectar-carbohydrate production between gland locations? All nine species possessed modified stomata on their nectaries, common in the Brassicaceae (Davis 1992; Deng and Hu 1995). Nectary stomata studied to date

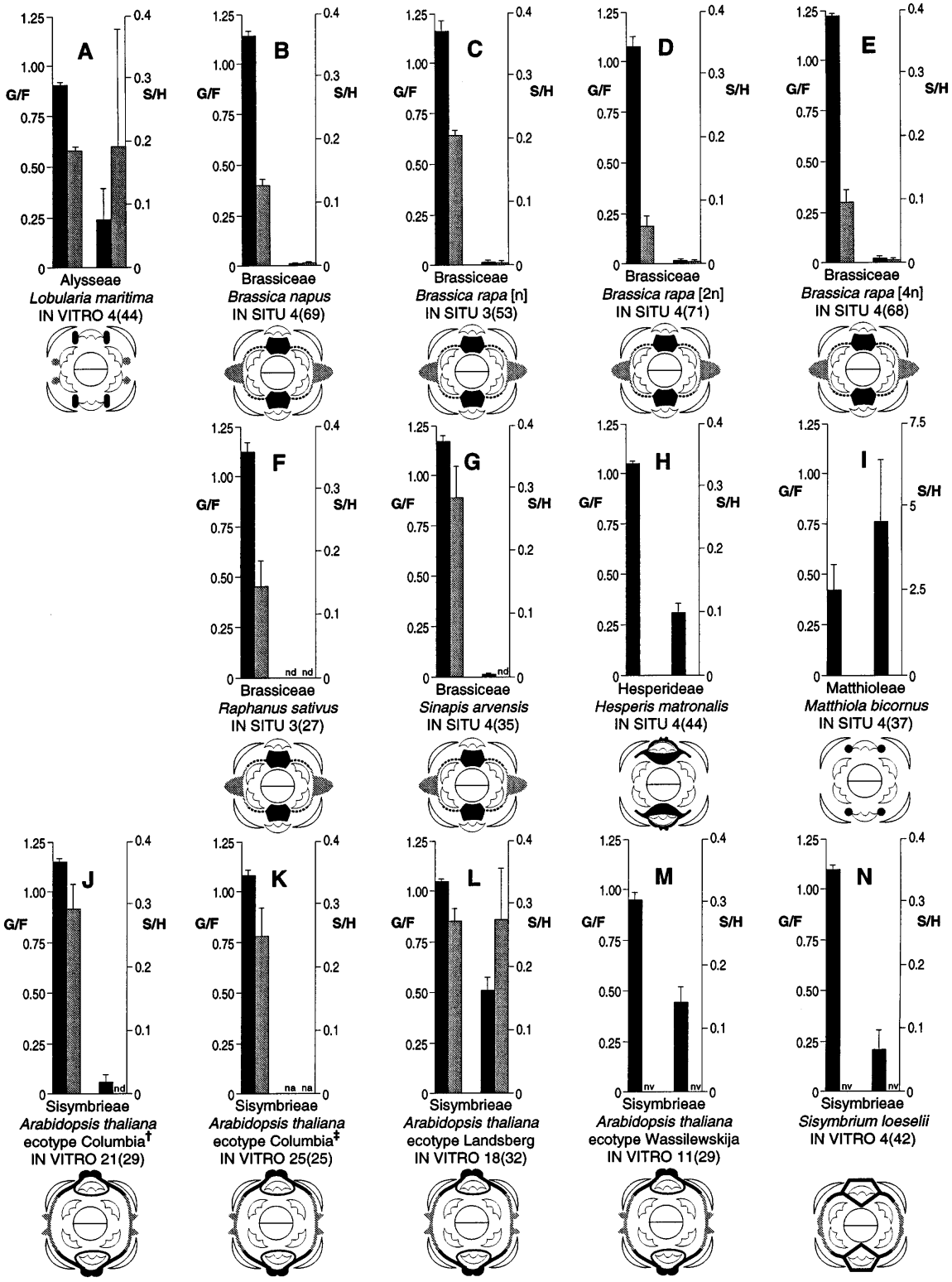


Fig. 5. Ratios of glucose/fructose (G/F) and sucrose/hexose (S/H) in nectar collected from lateral (*black bars*) and median (*grey bars*) nectaries. Numbers of plants (flowers) from which the nectar data were derived, are given. *na*, nectar not analysed for sucrose; *nd*, sucrose not detected; *nv*, nectar unavailable for analysis; †, HPLC analysis; ‡, enzymatic analysis

usually are considered "modified" (Fahn 1979; Davis 1997a) because they lack the ability to close their pores in the typical foliar fashion (see Davis and Gunning 1993 and references therein). The same situation apparently exists for these Brassicacean taxa. Relative fixation of the pore aperture seems to have a physical basis. Development of a relatively small substomatal space maintains firm contact between guard cells and subjacent

Table 3. Comparison of nectar-carbohydrate ratios (means ± SE) from lateral- and median-nectary locations from flowers on the plant (in situ) or cultured (in vitro). Within species, all flowers originated on the same plant

Species	Treatment	No. flowers	Glucose/fructose		Sucrose/hexose[a]	
			Lateral nectar	Median nectar	Lateral nectar	Median nectar
Lobularia maritima	In situ	6	0.77[a] ± 0.043	0.54[a] ± 0.047	0.059[a] ± 0.038	0[a]
	In vitro	11	0.87[a] ± 0.031	0.52[a] ± 0.026	0.0053[a] ± 0.0036	0[a]
Sisymbrium loeselii	In situ	8	1.10[a] ± 0.022	–[b]	0.0015[a] ± 0.0015	–[b]
	In vitro	11	1.09[a] ± 0.017	–[b]	0.018[a] ± 0.010	–[b]

[a]Within columns per species, nectar-carbohydrate ratios were not significantly different (Wilcoxon rank sum test, 2-sided, $P > 0.05$)
[b]Median nectar not available

parenchyma cells (Figs. 1G, 2J, 3E and M), unlike the leaf counterparts (F.A.I. Razem, Department of Biology, University of Saskatchewan; personal communication). Failure to finely control their pore apertures argues against a regulatory role for these structures during nectar secretion, as does the asynchrony in stomatal development found at the commencement of, and during, nectar secretion. Indeed, the small substomatal space may contribute to the high proportion of immature stomata detected (*R. sativus*, *M. bicornus*), by preventing the guard cells from expanding enough to ultimately tear the outer cuticle overlying an otherwise complete pore (Davis 1992; Davis and Gunning 1993). Perhaps it is not surprising then, that the average number of modified stomata on the lateral nectaries per flower did not correlate with nectar-carbohydrate production by the lateral nectaries. Neither did that relationship exist for the poorly yielding median glands. Collectively, the most extreme example occurred within flowers of *S. arvensis*, where median glands possessed 2.4-fold as many modified stomata as lateral ones, yet yielded only 8% of the total nectar sugar.

Phloem alone supplied the floral nectaries of all species studied here, a feature of the Brassicaceae (Fahn 1979) now upheld in over 40 species (Davis 1992). Whereas nectar-carbohydrate production is unrelated to the number of modified stomata per nectary, it is linked closely with the abundance of phloem supplying each nectary type. Median nectaries of the seven species possessing them received a comparatively small number of sieve tubes that, moreover, usually did not penetrate more than a few cell layers into the gland interior. On the other hand, lateral nectaries were supplied with several long strands of phloem that richly innervated them. In accordance with the relative phloem supply received, lateral nectaries produced 95% (on average) of the total nectar carbohydrate per flower. This finding supports the conclusion of Norris (1941) that nectarial tissue "without evidence of secretion was generally without any such strands". Moreover, the inferior nectar-carbohydrate production by flowers of *H. matronalis*, compared to their moderately large size, may relate to the relative scarcity of phloem that entered their lateral nectaries.

The significantly higher nectar-carbohydrate production in flowers of *A. thaliana* and *L. maritima* when cultured singly *ad libitum* suggests an intrinsic competition for limited photosynthate among the many fruits, flowers and buds within an inflorescence. By contrast, in *S. loeselii*, flowers in situ produced as much nectar carbohydrate as cultured ones. Absence of nectar outside the long stamens of this species, despite an abundant phloem supply to the nectarial tissue there, high humidity during culture, and the possession of distinct recesses in the petal claws opposite (identical to those facing the lateral-nectary apices, where nectar droplets were indeed cradled in the claw recesses), is difficult to explain. Modified stomata were never observed at those median-nectary positions, however, and might have facilitated nectar passage to the outside.

That large interplant differences in total nectar-carbohydrate production per flower can occur within species is well known (Cruden et al. 1983) and of breeding interest (Shuel 1989). Here, fewer than five unselected plants were investigated per species, with highest-producing plants yielding 2.3-fold more nectar carbohydrate on average than the lowest. This range agrees with other studies of Brassicaceae (Shuel 1989).

Nectar composition and production and their relationship to flower visitation and breeding systems. Glucose and fructose predominated in all floral nectars except that of *M. bicornus*. Therefore, even when median nectar was available and its relatively higher sucrose/hexose ratio factored into a species' overall nectar composition, the small-flowered taxa *L. maritima* (Fig. 5A) and ecotypes Landsberg and Wassilewskija of *A. thaliana* (Fig. 5L, M) would be designated marginally as "hexose-rich" ($0.499 < S/H$ ratio < 0.1), and all others (Fig. 5B–H, J,N) as "hexose-dominant" ($S/H < 0.1$) (sensu Baker and Baker 1983). Our results concur with the paper-chromatographic and other analyses in the literature for nectar of *B. napus*, *B. rapa*, *H. matronalis*, *R. sativus* and *S. arvensis*. Though not distinguished, it can be assumed that these samples were from the lateral nectaries only, or were a mixture from both gland types. Moreover, we confirm the "sucrose dominance" ($S/H > 0.999$) of *M. bicornus* nectar categorized as "SFG" by Percival (1961), it being the only one of 37 Brassicaceaen species to have appreciable quantities of the disaccharide in nectar. In several of those species, Percival (1961) failed to detect any sucrose in nectar, just as we did for *R. sativus*.

The composition of nectar and the quantities of nectar-carbohydrate produced by flowers in this study, both reflect an attractiveness to insects. Based partially

on Brassicacean taxa they surveyed, Baker and Baker (1983) reported that flowers regularly attended by flies (order Diptera), short-tongued bees (Hymenoptera) and butterflies (Lepidoptera), generally possess hexose-rich or -dominant nectar. In fact, Knuth (1908) recorded only insects, indeed belonging to these three orders and Coleoptera (beetles), as visitors to flowers of *A. thaliana*, *B. napus*, *B. rapa*, *H. matronalis*, *R. sativus* and *S. arvensis*. During nectar collection we regularly found thrips (Thysanoptera) at the flower bases. The quantities of floral nectar carbohydrate (0.0011–0.386 mg) per studied species also reflect bee and butterfly affinities (Cruden et al. 1983). Also, it might be predicted that, with their petals light-purple and nectar being well-concealed (Knuth 1908), sucrose-dominant (Baker and Baker 1983) and available in small quantities (Cruden et al. 1983), and with a strong fragrance released during anthesis at dusk, flowers of *M. bicornus* may be visited by nocturnal moths.

In the six species that provided nectar from both lateral- and median-nectary locations of the same flower, a consistent difference was detected in nectar-carbohydrate composition. Lateral nectar contained higher quantities of glucose than fructose. In contrast, the fructose content of median nectar was higher than glucose. Within each species, the glucose/fructose ratio in nectar from the lateral glands always averaged higher than that from the median nectaries. This striking difference was maintained throughout this study regardless of a wide range in (i) ploidy level (n–4n in *B. rapa*), (ii) flower size, (iii) nectar-production capacity, even when flowers were cultured, and (iv) nectary anatomy. Even the serendipitous example of Plant 1 of *S. arvensis*, wherein the glucose/fructose ratio of median nectar (Fig. 4F) was reversed from the norm (Fig. 5G and other species), validates the conclusion that these two nectary locations – within the same flower – exhibit their own distinctive, and independent, secretory metabolism.

This disparity in nectar-carbohydrate production and composition with nectary location in the cruciferous flower may have functional significance for pollination, but none could be ascribed. Five species that possess both lateral and median floral nectaries [*A. thaliana*, *B. rapa* (= *B. campestris*), *L. maritima*, *R. sativus*, *S. arvensis*] were found to produce similar quantities of pollen per anther in their long (median) and short (lateral) stamens (Preston 1986). Therefore, the greater quantity of nectar sugar produced at the base of the short stamens seems unrelated to total pollen availability per anther. Also, pollen viability in *A. thaliana* does not differ between long and short stamens (J.D. Pylatuik, Department of Biology, University of Saskatchewan; personal communication). Moreover, flowers possess functional nectaries yielding disparate hexose ratios regardless of the species' breeding system; both *A. thaliana* and *B. napus* are autogamous, and so do not require flower visitors to achieve seed set. *Brassica rapa*, *L. maritima*, *R. sativus* and *S. arvensis*, however, are allogamous (Preston 1986). It is possible that systems of incompatibility in the Brassicaceae arose secondarily, after evolution of floral nectaries.

Little evidence was found from the literature to suggest that various insect groups might be attracted differentially to the disparate nectar compositions offered by each nectary type within the same flower. Unfortunately, the lack of agreement between feeding experiments aimed at determining carbohydrate preferences within insect species (e.g., the honey bee, *Apis mellifera*; see Baker and Baker 1983), confounds the issue. Furthermore, despite their different proportions of glucose and fructose, by themselves the lateral and median nectars would both still be considered hexose-dominant (sensu Baker and Baker 1983). Nevertheless, observations of foraging on *B. napus* revealed that certain bees (*A. mellifera*, *Osmia* spp.) regularly collected nectar only from the lateral nectaries (Beutler 1930; Meyerhoff 1958; Eisikowitch 1981). Bumble bees, however, always probed both lateral and median nectaries (Eisikowitch 1981) and the presence of even some nectar at the median positions may facilitate contact with the anthers of the long stamens (e.g., *L. maritima*; Jordan 1886). Eisikowitch (1981) suggested, however, that preferential collection from the lateral nectaries may simply reflect the greater quantities of nectar there. Clearly, more attention to this question is required. Whether anthers open introrsely or extrorsely, or stamens shift position after anthesis (Jordan 1886; Eisikowitch 1981), also are noteworthy for pollination. Likewise, beyond the simple carbohydrates investigated here, knowledge of differences in other constituents (Baker and Baker 1983) between lateral and median nectar could shed more light on any evolutionary significance of possessing two nectary types per flower.

The observations that in *B. napus*, median nectar becomes available only at a later stage of anthesis and may reach a significantly higher concentration than lateral nectar (Eisikowitch 1981), further emphasizes the independence of the nectary systems per flower and raises the possibility of a different function for each nectary type. We have no evidence for this idea, but because nectaries of the Brassicaceae persisted (unlike other floral parts) below the expanding capsule, it is conceivable that some floral glands in that family serve a post-flowering defensive role, similar to the attraction of ants to the bases of fruits of *Mentzelia nuda* (Keeler 1981) and *Ruellia radicans* (Gracie 1991). Generally, droplets of hexose-rich nectars evaporate slowly (Corbet et al. 1979), a characteristic of importance when nectaries become fully exposed due to abscission of surrounding parts. In our studies, however, persisting nectaries barely glistened, and at least in *A. thaliana*, degenerated to a large extent before silique maturation (Davis 1992). Uncollected nectar residing on both nectary types eventually was resorbed.

Nectary diversity and the concept of "nectarium". Despite a remarkable conservation of basic floral form in the Brassicaceae (Endress 1992), an equally remarkable diversity in morphology, number and location of nectaries exists (Knuth 1908), as partially displayed in our study. Chemical analyses of the secretory products

of these nectaries, as well as developmental and anatomical data presented here and elsewhere (Davis 1992; Davis et al. 1996) clearly indicate that, even when nectarial tissue is united around the receptacle, there are actually two nectary systems present. The existence of this bilateral arrangement of nectaries accords with the established pattern seen in stamens and sepals of many Brassicaceae (Endress 1992; Davis 1997b). Whereas the nectaries have an obvious functional association at the base of the former, the lateral sepals particularly are often saccate and serve as reservoirs for accumulating nectar in several species studied here (Bayer 1905; Knuth 1908; von Hayek 1911; Schulz 1936). In *L. maritima* no major differences in sepal morphology occur (Fig. 1C, and Schulz 1936), nor in *S. loeselii* (von Hayek 1911) where the petals, not the sepals, cradle the nectar.

Just as "gynoecium" is used to refer collectively to all carpels in a flower, and "androecium" similarly used for all stamens, we propose (Davis 1997b) resurrection of the term "*nectarium*" (Linnaeus, cited by Fahn 1979) to represent all the nectarial tissue within each flower of the Brassicaceae. The term has application when referring to nectarial tissue at both lateral and median positions that is connected, such that the borders between "individual glands (can) not be sharply defined" (Arber 1931). In our study, such connections were universal in flowers of *S. loeselii* and common in *R. sativus*, but more sporadic or non-existent in the others. A relationship between frequency of these connections and ploidy was determined in *B. rapa* (Davis et al. 1996), perhaps indicative of differential spacing more-or-less established between petal and stamen bases by the time nectary enlargement finally occurred. "Nectarium" represents all nectaries abstractly, whether such connections exist or not; "nectary", being interchangable with "nectar gland" (Schmid 1988), represents a tangible entity having distinct boundaries.

Future value of the Brassicaceae for nectary and nectar research. Our growing understanding of the control of nectarial development at the genetic and molecular level (Davis 1994; Creemers-Molenaar et al. 1996; Baum et al. 1997) promises to provide significant insights into the evolution of the fascinating plant-animal symbiosis leading to plant reproduction via biotic pollinators. In *A. thaliana*, nectarial tissue, unlike other parts, has been detected inside homeotic-mutant flowers representing all three classes of the current ABC model of flower development (Davis et al. 1993). Accordingly, refinement to the model is required to explain the governance of nectarial tissue development. Hence, the relatively complicated possession of two nectary types in many Brassicaceaen species, like *A. thaliana*, provides an extra dimension to our search for a more complete understanding of basic flower development. For the Brassicaceae, uncovering of the molecular basis for nectary development could help to explain why half of all species lack median nectaries (Schweidler 1911), and why this gland type occurs in some members of a genus and not others (e.g., *Brassica balearica* – Hildebrand 1879; Clemente Munoz and Fernandez Bermejo 1978).

Owing to the importance of nectar chemistry to biotic pollination and honey stability, perhaps equally significant to uncovering the molecular basis for nectary development will be deciphering the biochemical mechanisms responsible for final nectar composition. To delay honey crystalization, such knowledge eventually could allow experimentation wherein the natural state is reversed, such that the relatively abundant lateral nectar yielded a lower glucose/fructose ratio (see *Introduction*) typical of median nectar throughout this study. Also, although hexoses appear to predominate in most nectars of the Brassicaceae, the availability of at least one species (*M. bicornus*) with sucrose-dominant nectar permits the family to serve as a suitable model group for exploring the biochemistry of a wide range of nectar compositions secreted by angiosperm taxa.

It is a pleasure to thank Prof. B.E.S. Gunning, The Australian National University, in whose laboratory the initial experiments were conducted, and Dr. P. Macnicol, C.S.I.R.O. Division of Primary Industry, who provided a most helpful introduction to enzymatic analysis of nectar. We appreciated the helpful critiques of an earlier version of the manuscript by Prof. Emeritus R.W. Shuel and two anonymous reviewers. Our thanks also are extended to Saskatoon contributions by Dr. A.M.R. Ferrie, Kristjanson Biotechnology Laboratory, for donating the haploid plants of *B. rapa*; Prof. Emeritus V.L. Harms, The W.P. Fraser Herbarium, for helping to identify the weedy species; Mr. D. Dyck for preparing Fig. 5; and Mrs. J. Smith and Mr. J. Sullivan for maintaining the growth-chamber plants. The Nottingham Arabidopsis Stock Centre donated the seed of *A. thaliana*. NSERC Undergraduate Student Awards (1995) to J.D.P. and J.C.P. and Research Grants to A.R.D. and N.H.L. provided the necessary funding, for which we are grateful.

References

Arber A (1931) Studies in floral morphology. I. On some structural features of the cruciferous flower. New Phytol 30: 11–41

Baker HG, Baker I (1983) A brief historical review of the chemistry of floral nectar. In: Bentley B, Elias TS (eds) The biology of nectaries. Columbia University Press, New York, pp 126–152

Baum SF, Alvarez J, Bowman JL (1997) Genetic analysis of nectary development. Abstract No. 3-2, 8th International Arabidopsis Conference, Madison, Wisconsin, USA, June 25–29, 1997

Bayer A (1905) Beiträge zur systematischen Gliederung der *Cruciferen*. Beih Bot Centralblatt 18(2): 119–180

Beutler R (1930) Biologisch-chemische Untersuchungen am Nektar von Immenblumen. Zeitschrift für Vergleichende Physiologie 12: 72–176

Clemente Munoz M, Hernandez Bermejo JE (1978) El aparato nectarigeno en la tribu *Brassiceae* (*Cruciferae*). Anal Inst Bot Cavanilles 35: 279–296

Corbet SA, Willmer PG, Beament JWL, Unwin DM, Prys-Jones OE (1979) Post-secretory determinants of sugar concentration in nectar. Plant Cell Environ 2: 293–308

Creemers-Molenaar J, Angenent GC, Yaxin G, Dons JJM (1996) Improvement of nectar quality by genetic modification. [Abstr] Meeting of the Nectar Working Group, International Commission for Plant-Bee Relationships, Lethbridge, Alberta, Canada, June 21, 1996

Cruden RW, Hermann SM, Peterson S (1983) Patterns of nectar production and plant-pollinator coevolution. In: Bentley B, Elias TS (eds) The biology of nectaries. Columbia University Press, New York, pp 80–125

Dafni A, Werker E (1982) Pollination ecology of *Sternbergia clusiana* (Ker-Gawler) Spreng. (Amaryllidaceae). New Phytol 91: 571–577

Daumann E (1931) Zur Phylogenie der Diskusbildungen. Beiträge zur Kenntnis der Nektarien. II. (Hydrocharis, Sagittaria, Sagina). Beih Bot Centralblatt 48: 183–208

Davis AR (1992) Physiological and structural aspects of floral nectar secretion. Ph.D. thesis, The Australian National University, Canberra, Australia

Davis AR (1994) Features of the nectary in wild-type and some mutant flowers of *Arabidopsis thaliana* (L.) Heynh. var. Columbia. In: Bowman J (ed) Arabidopsis – An atlas of morphology and development. Springer, New York, pp 172–177, 224–225

Davis AR (1997a) Influence of floral visitation on nectar-sugar composition and nectary surface changes in *Eucalyptus*. Apidologie 28: 27–42

Davis AR (1997b) And the nectaries make *two* more ... *eight* sets of floral parts in the wild-type flower of *Arabidopsis thaliana*. [Abstr No 115] Joint Annual Meetings of the Canadian Botanical Association/Botanical Society of America, August 2–7, 1997, Montreal, Quebec, Am J Bot, Suppl 84: 40

Davis AR, Gunning (1993) The modified stomata of the floral nectary of *Vicia faba* L. 3. Physiological aspects, including comparisons with foliar stomata. Bot Acta 105: 241–253

Davis AR, Haughn GW, Sawhney VK (1993) Preliminary investigation of the nectary in floral mutants of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). In: Veeresh GK, Uma Shaanker R, Ganeshaiah KN (eds) Pollination in the tropics. Proc Int Symp Polln Trop, August 8–13, 1993, Bangalore, India, pp 9–13

Davis AR, Sawhney VK, Fowke LC, Low NH (1994) Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). I. Nectary size and nectar carbohydrate production and composition. Apidologie 25: 602–614

Davis AR, Fowke LC, Sawhney VK, Low NH (1996) Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). II. Quantified variability of nectary structure and function in rapid-cycling lines. Ann Bot 77: 223–234

Deng Y-b, Hu Z-h (1995) The comparative morphology of the floral nectaries of Cruciferae. Acta Phytotax Sin 33: 209–220

Dvorák F (1967) A contribution to the study of the variability of the nectaries in the plants of the family *Brassicaceae*. Fac Sci Univ JE Purkyne, Brno 321–325

Dvorák F (1968) A contribution to the study of the variability of the nectaries. Preslia (Praha) 40: 13–17

Eisikowitch D (1981) Some aspects of pollination of oilseed rape (*Brassica napus* L.). J Agric Sci 96: 321–326

Endress P (1992) Evolution and floral diversity: the phylogenetic surroundings of *Arabidopsis* and *Antirrhinum*. Int J Plant Sci 153: S106–S122

Fahn A (1979) Secretory tissues in plants. Academic Press, London

Gracie C (1991) Observation of dual function of nectaries in *Ruellia radicans* (Nees) Lindau (Acanthaceae). Bull Torrey Bot Club 118: 188–190

Hildebrand F (1879) Vergleichende Untersuchungen über die Saftdrüsen der Cruciferen. Jahrbücher für wissenschaftliche Botanik (Leipzig) 12: 10–40

Jordan KF (1886) Die Stellung der Honigbehalter und der Befruchtungswerkzüge in den Blumen. Flora 69: 95–225, 243–252, 259–274

Keeler KH (1981) Function of *Mentzelia nuda* (Loasaceae) postfloral nectaries in seed defense. Am J Bot 68(2): 295–299

Kevan PG, Lee H, Shuel RW (1991) Sugar ratios in nectars of varieties of canola (*Brassica napus*). J Apic Res 30: 99–102

Knuth P (1908) Handbook of flower pollination. Volume 2 (Cruciferae). Oxford, Clarendon, pp 74–128

Kunze H (1995) Floral morphology of some Gonolobeae (Asclepiadaceae). Bot Jahrb Syst 117(1/2): 211–238

Low NH, Nelson DL, Sporns P (1988) Carbohydrate analysis of Western Canadian honeys and their nectar sources to determine the origin of honey oligosaccharides. J Apic Res 27: 245–251

McKenna M, Thomson JD (1988) A technique for sampling and measuring small amounts of floral nectar. Ecology 69: 1036–1037

Meyerhoff G (1958) Zum Sammelverhalten der Bienen in Raps. Leipzig Bienenzeitung 72: 164–165

Norris T (1941) Torus anatomy and nectary characteristics as phylogenetic criteria in the Rhoeadales. Am J Bot 28: 101–113

Percival MS (1961) Types of nectar in angiosperms. New Phytol 60: 235–281

Preston RE (1986) Pollen-ovule ratios in the Cruciferae. Am J Bot 73: 1732–1740

Schmid R (1988) Reproductive versus extra-reproductive nectaries – historical perspective and terminological recommendations. Bot Rev 54: 179–232

Schulz OE (1936) Cruciferae. Die natürlichen Pflanzenfamilien 17B: 227–658

Schweidler JH (1911) Über den Grundtypus und die systematische Bedeutung der Cruciferen-Nektarien I. Beih Bot Centralblatt 27(1): 337–390

Shuel RW (1989) Improving honey production through plant breeding. Bee Wld 70: 36–45

Velenovsky J (1883) O medových zlázkách rostlin krízatých. Ceské Spolecnosti Nauk. (K. Böhmische Gesellschaft der Wissenschaften) Prague. 12(7): 58 pp

von Hayek A (1911) Entwurf eines Cruciferen-Systems auf phylogenetischer Grundlage. Beih Bot Centralblatt 27: 127–335