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P. F. Torchio

Bee Biology and Systematics Laboratory, USDA-ARS, Utah State University, Logan, Utah 84322

G. E. Trostle

Bee Biology and Systematics Laboratory, USDA-ARS, Utah State University, Logan, Utah 84322

D. J. Burdick

Department of Biology, California State University, Fresno, Calif. 93710

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The Nesting Biology of *Colletes kincaidii* Cockerell (Hymenoptera: Colletidae) and Development of Its Immature Forms

P. F. TORCHIO,¹ G. E. TROSTLE,¹ and D. J. BURDICK²

Ann. Entomol. Soc. Am. 81(4): 605-625 (1988)

ABSTRACT Overwintering larvae of *Colletes kincaidii* Cockerell were obtained by removing soil blocks from a large nesting site established in a hardened, vertical sandstone cliff at Bonny Doon, Calif. One sandstone block was placed in a greenhouse at Logan, Utah, where two emerging females established nests within glass tubes inserted into observation boxes and one female excavated a nest in soil beneath the sandstone block from which she emerged. Observations of nesting biology in glass tube nests showed for the first time that polyester cell linings are composed of a mixture of salivary and Dufour's gland materials. Salivary secretion is first brushed onto nest walls with the glossa; Dufour's gland liquid is deposited on top of the salivary layer and brushed into a thin cover layer with the bifid glossa. The careful sequence of glandular deposition, combined with physical evidence obtained during observations, suggests that salivary gland material may carry an enzyme that links lactones derived from the Dufour's gland into polyester chains that are incorporated into cell linings. Cell platform construction and how this platform is used to position the female during the act of egg deposition are described. The physical restructuring of the platform into a cell cap immediately after the egg is deposited is also described. Cell provisioning and methods used to deposit the egg are described in detail. Late embryogenesis and immature development were observed in the laboratory using in vivo and in vitro methods. These observations confirm the 180° rotation of the embryo, eclosion from the egg chorion as a second instar, anus-to-mouth feeding habits of the larva, and the migration pattern of the larva from its position within the egg to the cup-shaped central position on the surface of the cell provisions. Supportive studies in the field confirmed that some females in a nesting population excavate their entire burrow system while others use abandoned nests and extend lateral burrows before constructing cell series in these new excavations; some females use abandoned nests to construct cell series only within the residue cell linings produced by previous generations. These studies also confirmed that any one cell of *C. kincaidii* is provisioned with only one pollen host species, but any number of pollen host species can be used by the same individual, different individuals, or populations that are distributed across North America; and that *Epeolus compactus* Cresson and *Paravilla fumosa* Hall were found parasitizing *C. kincaidii*. Much of the new information is used as a first step in establishing the evolutionary status of Colletidae.

KEY WORDS Insecta, biology, embryogenesis, phylogenetics

Colletes IS A SPECIES-RICH GENUS of bees that is distributed worldwide except for Australia. These bees are moderate to large in size, and they nest in soils usually devoid of plant growth. Most nesting sites are perennial, each supporting a large annual aggregation of nesting females. Because of these characteristics, many nest sites of *Colletes* have been found, and the biology of some species has been studied.

Kirby (1802) was first to publish on the biology of *Colletes*; approximately 23 additional species have been studied since. None of these species has been observed throughout an entire nesting period and, therefore, much of our biological understanding of the genus is based on short notations in the

literature. Stephen (1954) summarized the bionomics of this genus, and Torchio (1965) updated a review of the literature. More recent studies on *Colletes* biology include Rozen & Favreau (1968), Tsuneki (1970), Grewal et al. (1970), Rajotte (1979), Batra (1977, 1980), and Albans et al. (1980).

Combined, these studies suggest that *Colletes* species express a wide range of behavioral and biological characteristics that are species specific (Stephen 1954). Architectural features of nests and cell structures, however, are shared by many of the congeners with little interspecific variation. Consequently, it is presumed that all *Colletes* follow the same or similar behavioral patterns in producing the diagnostic polyester cell linings at or near the terminus of excavated burrows.

Until now, no one has had the opportunity to observe the in-nest biology and immature development of any *Colletes* species. Yet the bionomics of this genus is often used in comparative studies

¹ Bee Biology and Systematics Laboratory, USDA-ARS, Utah State University, Logan, Utah 84322.

² Department of Biology, California State University, Fresno, Calif. 93710.

that emphasize the phylogenetic status of Apoidea, the Colletidae or both (Michener 1953, Malyshev 1968, Iwata 1976, Rozen 1984, Torchio 1984). Herein, we give a detailed account of direct observations on the nesting biology and immature development of *Colletes kincaidii* Cockerell for two reasons: to contribute to the general understanding of *Colletes* biology and to strengthen the biological bases on which future phylogenetic studies will stand.

Bionomics

Nest Sites. The field nest site was located at Bonny Doon, Santa Cruz County, Calif. This mostly flat terrain is interrupted by a number of monolithic, hard-cased, eroded sandstone cliffs in which *Colletes kincaidii* nests. The tallest cliffs are 95 m; they support small to large aggregations of nesting populations (20–1,500 individuals) that have used these sites for at least 110 continuous years (A. Moldenke, personal communication). The climax forest of this inner coastal region is composed primarily of *Pinus ponderosa* Lawson, *P. attenuata* Lemmon, and *Lithocarpus densiflora* (H. and A.) Rehd. *Arctostaphylos* is the dominant shrub growing adjacent to nest sites, and bear grass, *Xerophyllum tenax* (Pursh) Nutt., is the dominant plant found in the few open and disturbed areas between the cliffs.

In early March 1985, two large blocks of sandstone containing live nests of *C. kincaidii* were removed from one of the Bonny Doon cliffs and transported to the USDA laboratory in Logan, Utah. One of the blocks was dissected in mid-March to decipher nest architecture and to obtain overwintering prepupae. These larvae were weighed, inserted individually into Beam capsules (Better Equipment for Electron Microscopy, Bronx, N.Y.), and placed in a controlled-temperature cabinet (3°C) on 18 March. The capsules were moved to a 26°C cabinet on 18 June 1985. The encapsulated larvae molted into pupae between 1 and 5 July, and adults (males followed by females) emerged from 15–19 July 1985. Each adult was weighed 24 h after emergence, and it was then released into the greenhouse. The coloring sequence of the developing adult encased by pupal cuticle is outlined below, and results obtained from larval-adult weights are included in the discussion.

The second block of sandstone was not dissected because of time constraints, but it was retained in the laboratory and exposed to room temperatures (18–23°C). On 30 May 1985, four male *C. kincaidii* emerged from this sandstone block, and we moved these bees and the sandstone block into a greenhouse supplied with a variety of flowers in bloom—*Phacelia tanacetifolia* Benth., *Melilotus alba* Desc., *Coreopsis atkinsonia* Douglas, *Tithonia rotundifolia* (Mill.) S. F. Blake, *Centaurea cyanus* L., *Oenothera hookeri* T. and G. An unknown number of bees emerged from the soil block during

the next 2 d, and both sexes were observed collecting nectar from all of the available flowers throughout the following week. On 14 June, 1 of 11 *C. kincaidii* females flying in the greenhouse initiated nesting in one of many glass tubes inserted in an observation box (see Torchio 1972, 1984 for detailed descriptions) originally designed to observe the nesting biologies of megachilid bees. A second female accepted a glass tube positioned immediately below the first female's nest on 16 June. On 18 June, a third female initiated a nest burrow in moist, black soil directly beneath the block of case-hardened sandstone from which she emerged. No other *C. kincaidii* females nested in the greenhouse during the 1985 flight season.

Nest Architecture. We made a return trip to the Bonny Doon site on 5–6 July 1985 to observe adult activities during peak nesting periods and to obtain live eggs and young larvae of the cleptoparasitic bee, *Epeolus compactus* Cresson. We excavated many active and inactive nests from the base of one sandstone cliff during this period and compared the results from these examinations with those obtained from the block of sandstone dissected 3 mo earlier (March).

These combined results indicated that the architectural design of Bonny Doon *C. kincaidii* nests followed a basic pattern: (1) The nest entrance was at a right angle to the vertical face of the cliff, and the unlined, unplugged, horizontal, linear, or non-linear main burrow (5–8 mm diameter) penetrated the sandstone to a depth of 7–15 cm before it turned sharply to one side or the other and ended abruptly 2–6 cm beyond the bend. (2) One to three lateral burrows arose at or near the terminus of the main burrow; each was unlined, of the same diameter as the main burrow, straight or curved, and 3–11 cm long. In addition, each lateral burrow followed a different direction as it angled away from the main burrow, but all laterals were excavated on a horizontal or nearly horizontal plane. (3) Translucent, polyester-lined cells, each with a cell cap angled at 60–70° above horizontal, were constructed in linear series within the lateral burrows. These cells (2–11 per series) were firmly bound together (cell cap to basal partition) and positioned on or near the horizontal. In some nests, the last constructed cell of each series was covered by an elongate, cell-like structure composed of a thick layering of cell lining material that loosely covered the burrow walls. This "false cell" was firmly attached across the entire cell cap surface of the outermost cell. Variable quantities of dry pollen often were deposited within this 11–24-mm tube-like structure. (4) The main burrow, and those sections of each lateral burrow not filled with cells, remained open throughout the year. (5) Polyester nest plugs were constructed near the surface of the main burrow but only in nests with "false cells." (6) Some nests were freshly excavated by females; each appeared to be used by the same female throughout the nesting period. Abandoned nests of

one or more years old were used by other females, who lengthened extant lateral burrows or excavated new lateral burrows before constructing cell series in the freshly excavated burrow extensions. Other abandoned nests were used by females who did not extend burrows but constructed cell series only within the residual cell linings produced by previous generations. One cell series excavated in 1985 was covered by five separate layers of residue cell linings.

The nest architecture of the only nest excavated in soil beneath the sandstone block in the greenhouse was almost identical to those examined at Bonny Doon: the linear main burrow angled downward for 13 cm, then turned sharply to one side, and ended 2 cm beyond the bend at a point where the three lateral burrows joined. These lateral burrows (4–11 cm long) were excavated along a horizontal plane, but each was curved in a particular direction. Cells were constructed in series (5 in the shortest burrow, 8 in a longer burrow, and 10 in the longest burrow) as described above, and the first constructed cell of each series abutted the terminus of the lateral burrow. The burrow system of this nest, unlike those at Bonny Doon, was filled to the nest entrance with loosely packed soil. In addition, the silkened, tubelike false cell that was commonly found attached to the last-formed cell in a series at Bonny Doon was not found in this greenhouse nest.

Each of the two females that nested in glass tubes completed two nests and began a third. The cell series constructed in these tubes were identical to those described above, and they filled the inner sections of the horizontal, glass tube nests. None of these cell series had a false cell attached to the last constructed cell, but each completed glass tube nest had a thin layer of translucent cell-lining material constructed across the diameter of the tube several millimeters inside the entrance. The female formed this "nest cap" by depositing a latticework of salivary strands across the diameter of the tube during a 10-min period. This was followed by the deposition of anal and salivary liquids on the latticework to form the clear surface sheeting. The female mixed these clear liquids (salivary and Dufour's gland secretions) and brushed them across the surface of the latticework with the glossa (as described below) during an additional 5-min period.

Each of the six glass tube nests was 7 mm inside diameter by 13 cm long. Each of the two nesting females used three adjacent tubes to complete nesting during a 6-wk period. One female produced 28 cells (11, 9, and 8 per series); the second female produced 27 cells (11, 9, and 7 per series).

Cell Provisions. A. Moldenke (personal communication) examined numerous scopal pollen loads of *C. kincaidii* females from several nesting populations in Santa Cruz County, Calif. (1975–79); the only pollen species recovered was that of *Lithocarpus* (Fagaceae). We examined 32 cell provisions and 30 scopal pollen loads from the Bonny Doon

population and obtained the same data. We also found both sexes of *C. kincaidii* visiting only flowers of *Lithocarpus* at the Bonny Doon site on 5–6 July 1985.

When the three female *C. kincaidii* initiated nesting in the greenhouse, they collected only *Phacelia tanacetifolia* (Hydrophyllaceae) pollen during early cell provisioning activities. However, as nesting progressed, the flowering cycle of *Phacelia* peaked as *Melilotus alba* (Leguminosae) reached 35% bloom 8 d after the first *C. kincaidii* female began to provision cells. Soon afterward (4 d), both females nesting in glass tubes began collecting pollen only from *M. alba*. This switch in pollen species occurred within a 24-h period when each female began to provision a freshly lined cell. The same females then used 100% *M. alba* pollen to provision all of the remaining cells constructed during the 1985 season. The abrupt changes made in using different pollen species did not influence the average number of collecting trips to provision a cell (10), the in-nest and out-of-nest time required for each provisioning trip (see Cell Provisioning), or the survival of developing immatures (100%). In fact, the rate of cell construction (one cell per flight day) was maintained by the two females using the glass tube nests throughout the nesting season irrespective of which pollen species was collected.

1986 Study. The discovery that some individuals in one population of *C. kincaidii* established nests in preexisting cavities and others excavated nests directly into case-hardened sandstone was one of the more interesting observations made during the 1985 study. We also learned that the rate of parasitism by *Epeolus* was four times higher in nonexcavated host cell series constructed within residual cell linings compared with cell series constructed in freshly excavated burrows (Torchio & Burdick 1988).

These results prompted us to ask whether female progeny from cell series constructed in preexisting cavities and from excavated nests inherit the same nest choice habits expressed by their female parents. To answer this question, six male and six female *C. kincaidii* (reared from the 1985 nest excavated from beneath the block of sandstone placed in the greenhouse) were released on 3 June 1986 in the greenhouse, which was supplied with the same materials that had been used in 1985. Two females established nests in glass tubes 4 d after their release. The remaining bees continued flying but never initiated nesting. During the following week, each nesting female constructed a cell lining and provisioned one cell before absconding. Unfortunately, a thrips infestation drastically reduced available bloom in the greenhouse during this period; consequently, all nesting activities (including nesting by a few osmiine bees introduced earlier) were curtailed. These preliminary results suggest that nonheritable factors play an important role when individual *C. kincaidii* females select a nesting medium.

Fig. 1-6. Schematic diagrams showing sequence of cell constructing activities by *C. kincaidii* in longitudinal view. (1) Completed cell cap on which new cell is constructed in glass tube nest. (2) Basoventral angle formed in cell under construction and side wall coated with secretion. (3) Basal section of cell completely lined. (4) Cell walls lined. (5) Lump-like salivary elevations deposited on ventral surface of cell. (6) Posterior section of platform (PSP) constructed. (a, cell cap; b, deposition of Dufour's gland droplets; c, basoventral angle of cell lining; d, first glossal streaks of cell lining on cell wall; e, completed layer of cell lining covering cell cap of previous cell; f, lump-like elevations constructed with salivary material; g, posterior section of platform).

Cell Construction

We observed two female *C. kincaidii* continuously throughout the periods when three cells were constructed within glass tube nests in the greenhouse during June 1985. Periodically, we observed cell-constructing activities by these same females as they added six additional cells to their glass tube nests. The methods used to line the cells and the behavioral sequences required to complete the cell lining followed patterns that varied little from one female to another or between cells constructed by the same female. The following description of cell-lining activities is based on a composite of observations made during the construction of the nine cells.

After reshaping the cell cap of a completed cell (described below), the female moves close to the mouth of the glass tube where she rests for 4-24 h. She then crawls backward into the glass tube to a point just forward of the previously completed cell, extrudes small quantities of a clear glandular liquid (presumably Dufour's gland secretions) (Fig. 1, arrow b) from the tip of her abdomen and dabs this fluid onto the ventral surface of the tube (previously coated with salivary secretion as described below). During the next minute, she crawls forward a short distance, turns a half-somersault, extends her proboscis until the glossa is pressed to the cell

wall, and then walks forward toward the last completed cell as the glossa brushes salivary material onto the glass tube surface (Fig. 2, arrow d). To initiate each brush stroke, the female extends her proboscis until the glossa touches the substrate at a point below the front coxae. As the female begins to walk forward, the tip of the glossa bends posteriad, and the labial palpi trail behind the glossa. Salivary liquid is applied continuously as she walks forward, and this fluid solidifies on contact with the substrate.

The female stops walking when her proboscis touches the clear microdroplets (Fig. 1, arrow b) deposited less than 2 min earlier. At this point, she swings her head upward until the glossa touches the apex of the indented section of the cell cap that seals the last-constructed cell. The salivary material continues to flow from her mouth and onto the glossa as she lifts her proboscis from the substrate. The resulting mixture of abdominal and cephalic liquids rapidly solidifies into a narrow strip of cell-lining material that trails behind the glossa. These activities are repeated 6 to 12 times in the following minute before the female backs up a short distance, turns a half-somersault, and repeats the entire sequence of depositing the rapidly drying liquid secretions for an additional 3 min. One or more layers of cell lining material are formed during these

Fig. 7-12. Schematic diagrams showing sequence of cell constructing activities by *C. kincaidii* in longitudinal view. (7) Dorsal section of platform (DSP) constructed as an extension of the PSP. (8) Anterior section of platform (ASP) constructed as an extension of the DSP and melded to the ventral cell wall anteriorly. (9) Foundation of cell rim (upper arrow) formed and joined to the cell platform where the PSP and DSP meet (lower arrow); pollen grains (as dots) on ventral surface of cell. (10) Cell rim formed and pollen grains moved to base of cell. (11) Pollen grains at base of cell wetted with nectar. (12) Provisions moved by tip of abdomen to cover entire basal surface of cell. (g, posterior section of platform; h, dorsal section of platform; i, anterior section of platform; j, cell platform; k, cell rim).

activities, and each is melded to the preceding cell's cap and to the ventral surface of the glass tube to form the "basoventral angle" of the cell lining (Fig. 2, arrow c) under construction.

Immediately thereafter, the female moves the tip of her abdomen downward as she crawls backward into the glass tube until her midabdominal terga are pressed against the cell cap. She extends her proboscis, presses her bifid glossa to the ventral surface of the glass tube, and brushes salivary material onto that surface as she walks forward until her abdomen returns to a linear position. She repeats this procedure six to eight times in less than 1 min as parallel or overlapping streaks of salivary material are deposited across the floor of the anterior section of the developing cell lining. These activities end when, the female backs up to the cell cap and uses the tip of her abdomen to dab microdroplets of anal fluid along the edges of the basoventral angle of the cell under construction and along the lower edges of the cap of the previously completed cell. She then walks forward, turns a half-somersault, extends her proboscis, and again releases salivary liquid that is brushed onto the cell wall as she moves forward. She stops walking when her glossa touches the still-wet microdroplets deposited on the basoventral angle of the

cell under construction. She then swings her head upward to allow the glossa to continue its brush stroke through the microdroplets deposited on the edges of the cell cap. Normally, each glossal brush stroke follows the edge of the preceding cell's cap, but sometimes the glossa is directed toward the center of the cell cap after it passes through these droplets.

During the next 50 min, liquids issuing from the anal orifice are combined with salivary secretions as the female brushes cell-lining material across the entire anterior face of the previously completed cell and walls of the cell under construction (Fig. 2). This layering of cell lining is extended for distances of 1.5 to 3.0 body lengths forward of the basoventral angle. To accomplish this, the female positions her body at a different point in the cell after each half-somersault, and she always walks at an angle to the cell's linear axis as she brushes cell-lining material. As each half-somersault is made, the female extends her proboscis a short distance so that the glossa passes over the posterior tip of the abdomen and a small quantity of anal fluid (presumably Dufour's gland secretion) is transferred directly onto the glossa.

During this same time, liquids issuing from the anal orifice are dabbed periodically onto the outer

edges of the preceding cell's cap; they are then combined with salivary materials as the glossa is brushed across the face of the cell cap. As a result, a separate layer of cell-lining material is formed over the existing cell cap. This layer is melded to the basoventral angle, and together they form the entire basal section of the cell under construction (Fig. 3).

During the next 4 min, the female continues brushing cell-lining material across the surface of the forming cell (Fig. 4). However, these activities are modified periodically as the female deposits five or six, visible lumplike structures (composed of salivary secretion) (Fig. 5, arrow f) that are positioned in a straight line that traverses the ventral surface of the forming cell (Fig. 5). Each of these elevated structures is produced when the female turns the tip of her abdomen downward as she backs completely into the cell under construction, extends her proboscis, brushes salivary material onto the substrate as she walks forward, stops when her abdomen straightens, and begins bobbing her head up and down as salivary material continues to flow across the glossa. When the proboscis is lifted from the substrate during these bobbing motions, a strand of salivary material appears from the medial emargination on the glossa. As this pliable strand solidifies, it is pushed to the cell surface when the female bobs her head downward. The elevated structure takes form 20 s after the female begins these head-bobbing motions (Fig. 5).

During the next 25 min, the female works rapidly to construct the cell platform. She discontinues dabbing abdominal fluids onto cell surfaces, but she moves her antennae, maxillary palpi, and labial palpi very rapidly throughout this period. Thus, she continues adding salivary material to the cell walls, but she brushes the anterior section of the forming cell for longer periods (1.5 min) before turning half-somersaults and brushing the basal sections of the cell for 30-s periods.

Soon after these agitated activities begin, the female halts her forward progress whenever her glossa touches one of the elevated lumps on the ventral surface of the cell. She then lifts and swings her head to one side or the other while continuing to emit salivary secretion. This fluid flows down the proboscis and through the medial emargination of the glossa as the material hardens into a strand that tracks behind the proboscis.

The continuous strand of salivary secretion that is produced follows directly behind the glossa as the proboscis swings to one side until it touches the lateral section of the cell wall at a point in front of and above the elevated lump. Each time one of these elevations is touched by the glossa, a salivary strand is produced. These activities continue for as long as 3 min, until a latticework of salivary strands is constructed as a thin layer. This layer arises from the transverse line of elevations positioned across the ventral surface of the cell, and it extends upward at a 60–70° angle. The female then presses

her glossa firmly against the base of this latticework and, during the next 4 min, a matrix of salivary secretion is brushed across the surface of the latticework until a thin, unbroken layer of salivary secretion covers and is melded to these overlapping and interconnected salivary strands. This structure is named the posterior section of the platform (PSP) (Fig. 6, arrow g). The length of the female's tongue, combined with the specific upward and arching movement of her head during deposition of these salivary strands, establishes the 60–70° angle of the PSP.

A latticework of salivary strands is then added onto the front edge of the PSP during the next 3 min. This extension, called here the dorsal section of the platform (DSP) (Fig. 7, arrow h), is first dome shaped, but then it slopes downward as it extends forward some distance until it is melded to the ventral cell wall lining (the anterior section of the platform [ASP]). These three interconnected sections of latticework (PSP, DSP, ASP) form a large, ventral ridge at the anterior margin of the cell and together are named the cell platform (CP) (Fig. 8, arrow j).

The formation of the DSP is initiated when the female first walks onto the completed PSP layer, deposits salivary material as she moves forward, continues to emit salivary secretion after the proboscis is carried beyond the leading edge of the PSP, and swings her head sharply to one side or the other (not upward). As a result, a salivary strand is attached to the cell wall forward but laterad of the PSP.

As the dome of the DSP takes form (Fig. 7), the female walks onto the structure (PSP and DSP) at an angle as she brushes additional salivary material onto its surface. This allows her head to be positioned near the side wall of the cell when the proboscis is carried beyond the leading edge of the structure. The female (without turning her head to one side) continues moving forward as her extended proboscis is lifted to a nearly linear position. Thus, the salivary strand that tracks directly behind the glossa is attached forward of the DSP but slightly lower on the cell wall. These activities are repeated as the now downward sloping latticework is extended forward until it joins and is melded to the ventral cell wall lining. At this juncture, the framework of the ASP is complete (Fig. 8).

The female then begins to coat the DSP and ASP surfaces with a sheeting of salivary matrix in the same manner as before. After less than 1 min, she crawls backward a short distance, places her glossa on the lower surface of the cell, and brushes salivary material across its surface as she crawls forward at an angle. This brushing continues as she crawls onto the PSP surface, but her glossa soon touches the edge of the platform where the DSP and PSP surfaces join (Fig. 9, lower arrow). She then stops her forward movement and twists her head so that the extended proboscis follows an upward arc as the glossa continues to track across the

surface of the glass tube. At the same time, she moves her entire body around the circumference of the cell until the glossa encircles the glass tube and comes to rest on the opposite edge of the platform where the DSP and PSP surfaces join. These movements cause the glossa to circumscribe the inner surface of the tube as a continuum of the 60–70° angulation first established during the construction of the PSP. Only the lateral lobes of her glossa touch the glass tube while her proboscis circumscribes its surface; as a result, the salivary secretion flows through the bifid section of her glossa and is deposited as a continuous strand on the glass wall (Fig. 9, upper arrow). The female very rapidly repeats this procedure and deposits one or two additional parallel strands in a 30-s period.

During the next 1–2 h, the female continuously brushes salivary material on all surfaces of the cell as she faces the nest entrance. She adds salivary material to the entire surface of the platform and then adds another coating of salivary secretion on the cell walls. When her proboscis touches one of the parallel salivary strands that circumscribes the cell above the platform (Fig. 9, upper arrow), she reacts in the manner described above when her proboscis abutted the lumplike elevations. As a result, short, arching strands are attached to those two or three parallel strands laid down earlier, and this narrow band of latticework is eventually coated with a matrix of salivary secretion. This structure is subsequently used as a foundation on which the cell cap is bonded; it is therefore named the cell rim (Fig. 10, arrow k).

During this same period, the female periodically extends her proboscis slightly and brushes the glossa across the tip of the abdomen as she turns half-somersaults within the cell. She then brushes additional liquid materials (mostly salivary) onto the basal surface of the cell for short periods. Before one of these half-somersaults, she deposits a small quantity of anal fluid on the ventrobasal angle of the cell and brushes this liquid across that surface of the cell for a short time before returning to other duties.

At the end of this 1–2 h period, the female crawls to near the nest entrance and rests for at least 1–2 h. She then crawls back into the cell, periodically extends her sting from the abdominal tip, and walks forward as she wags the tip of her abdomen across the cell floor (including platform). She preens her entire body, crawls back into the cell, and repeats the abdominal-wagging cycle several times during an 8-min period (no visible materials are deposited during these activities). The female again returns to near the nest entrance and rests for an additional 2 h before leaving the nest to initiate cell-provisioning activities.

Cell Provisioning

On 18 June 1985, a female *C. kincaidii* was observed continuously while she provisioned a cell

in a glass tube nest. We made additional observations (23 cells) during the 1985 nesting period in the greenhouse to determine if cell-provisioning behavior varied with individual females or between cells constructed by the same female, or if behavior changes occurred through the nesting period. These observations demonstrated that cell-provisioning behavior varied little between individual bees or between cells constructed by the same bee. Therefore, we describe our observations on 18 June 1985, because they typify cell-provisioning behavior of this colletid species, and the results can be used as a standard for future comparative studies.

At 1605 hours (MDT), the female left her nest for the first time that day. She returned at 1620 hours with a load of dry yellow pollen on her hind legs and immediately crawled into the glass tube and then into the empty cell, where she tapped the basal surface of the cell with the tips of her antennae for 22 s. She backed out of the cell and turned a half-somersault within the glass tube so that she faced the nest entrance. She then backed into the cell until the tip of her abdomen touched the basal section of the cell. Pollen was subsequently removed from the hind legs as the midlegs moved alternately and repeatedly across the scopae of the hind legs. Most of the pollen grains fell onto the floor of the horizontal cell as a result of these brushing activities, which continued for 2.5 min (Fig. 9). Immediately thereafter, she walked forward to near the nest entrance, preened her entire body for 7 min; she used her hind legs to clean abdomen, her midlegs to clean thorax and wings, and her front legs to clean head and mouthparts. Then she rested for 4 min at the nest entrance.

The female left the nest at 1635 hours and returned 10 min later with a second pollen load. She repeated the behavioral sequences described for the first pollen load; but after depositing the pollen, she turned a half-somersault in the tube, crawled forward until she entered the cell, placed the distal tip of her labrum (fringed with long hairs) against the cell floor, and moved her head from side to side as she continued to crawl forward until her frons touched the basal section of the cell. As a result, most of the pollen previously deposited on the floor of the cell was pushed backwards by the brushing motions of the labrum (Fig. 10). The female then backed out of the cell, extended her proboscis, brushed additional salivary secretions across the ventral surface of the cell, crawled out of the cell, stopped near the nest entrance, preened for 2 min, and left the nest at 1710 hours.

She returned with a third load of pollen at 1717 hours and crawled head-first into the glass tube and then into the cell. She lifted her head slightly, spread her mandibles a short distance, and began flicking her proboscis in and out as nectar was disgorged from her mouth and onto the moving tongue. As nectar reached the glossa, she began to bob her head up and down rapidly for 20 s. This head

motion allowed the tip of the glossa to touch the provisions repeatedly as nectar was dabbed onto the surface of the pollen clump. The female then backed out of the cell, turned a half-somersault in the glass tube, and crawled backward into the cell where pollen was removed from her scopae as previously described. She then took two steps forward, bent her abdomen downward until its posterior tip touched the ventral surface of the cell at an angle of 20° above horizontal. Her abdomen then pumped rapidly; these movements allowed the tip of the abdomen to push most of the pollen on the cell floor onto the freshly wetted provisions (Fig. 11). The female then backed farther into the cell until the tip of her abdomen was immersed in cell provisions. Immediately thereafter, she began twisting her abdomen in a circular motion for 15 s; these movements allowed the tip of the abdomen to brush liquefied provisions across the entire basal surface of the cell (Fig. 12). The female crawled out of the cell, preened her entire body for 3 min, and left the nest at 1735 hours.

At 1740 hours the female returned with a fourth load of pollen. Upon entering the nest, she spread her mandibles and again used the labral brush to collect residue pollen on the cell floor as she moved forward. Then, tilting her head upward, she dabbed her labral brush into the provisions for 12 s as pollen grains were transferred from the labrum onto the wetted surface of the provisions. She then preened each mandible and the labrum with alternate stroking motions of the front legs for 32 s. Then she lifted her head, extended her tongue until the glossa was immersed in provisions, regurgitated nectar from the mouth and onto the proboscis, and moved her head in various directions for 20 s. This carried the proboscis through the provisions in a stirring motion as nectar was added. She crawled from the cell, cleaned her extended proboscis with alternate and scraping actions of the front legs (antennal cleaners used exclusively), turned a half-somersault in the glass tube, backed into the cell, and removed pollen from her scopae.

As this activity ended, the female abruptly moved a short distance forward (with midlegs folded on cell platform), rubbed her hind legs (tibiae and tarsae) together in an alternating, hand-washing motion. She then moved her hind legs (tibiae and tarsae) to the sides of her abdomen and reinitiated the hand-washing motion of the hind legs. These preening activities dislodged pollen grains from body hairs, but they fell on the cell floor rather than directly onto the wetted provisions spread across the base of the cell. The female then crawled forward into the glass tube where she spent 52 s cleaning her mouthparts by appressing the antennal cleaners of the front legs against the extended proboscis before moving her front legs in a hand-washing motion. She then crawled to near the nest entrance and rested for 2 min before leaving the nest at 1750 hours.

She returned to the nest with a fifth load of pollen

at 1755 hours and left the nest at 1802 hours. The methods used in depositing nectar and pollen and the sequence of these activities were identical to those described for depositing the fourth pollen load.

When the female entered the nest with a sixth load of pollen at 1810 hours, she crawled directly into the cell. She then extended her proboscis until the glossa was immersed in provisions, and she moved her tongue through this liquefied layer continuously for 3 min as regurgitated nectar flowed steadily down the proboscis and was added to the provisions. She then retracted her proboscis, backed out of the cell, turned a half-somersault, backed into the cell, deposited pollen as before, crawled forward, preened, rested, and left the nest at 1817 hours. Examination of the provisions during her absence revealed that most of the sixth pollen-nectar deposition was added to the ventral angle of the existing provision (Fig. 13).

Full pollen loads were carried to the nest during the next four provisioning trips. The female repeated the same in-nest activities as for the fifth and sixth provisioning trips. The time periods for each of these provisioning trips were as follows: 7th trip—entered nest at 1825 hours, left nest at 1829 hours; 8th trip—entered nest at 1834 hours, left nest at 1840 hours; 9th trip—entered nest at 1849 hours, left nest at 1904 hours; 10th trip—entered nest at 1910 hours, left nest at 1917 hours. After the 10th trip, the provision was 1.5 mm thick at its upper margin and 3 mm thick along its lower margin.

The female returned from her last (11th) provisioning trip at 1925 hours with a smaller quantity of scopal pollen. Upon entering the cell, she deposited a small quantity of nectar and then pollen on the provisions before crawling forward into the glass tube, where she preened her entire body almost continuously for 40 min. At 2015 hours the female backed into the cell and thereafter she moved methodically as she again rubbed the tip of her abdomen with her hind legs in a handwashing motion along the pleural regions. She crawled out of the cell at 2018 hours, turned a half-somersault in the glass tube, returned to the cell, and disgorged a large quantity of nectar during a 2-min period while rapidly moving her extended proboscis back and forth through the provisions. She again crawled out of the cell, turned a half-somersault, backed into the cell, and continued to preen the posterior tip of her abdomen. As preening continued, she flexed her abdomen until its posterior tip touched the cell lining immediately in front of the provisions. Then she pumped her abdomen rapidly and rotated it around the circumference of the cell. These concomitant activities served two primary functions: to sweep residue pollen grains from the cell walls onto the surface of provisions, and to tamp and better bind the outer edges of the provisions to the cell walls by pushing the tip of the abdomen into the provisions with each pumping

Fig. 13-18. Schematic diagrams showing sequence of cell provisioning, egg deposition, and cell closure by *C. kincaidii*. (13) Lower section of provisions extended beginning on 6th provisioning trip. (14) Provisions after 10th provisioning trip. (15) Egg attached to cell wall and Dufour's glandular droplets deposited adjacent to egg. (16) Cell platform disattached from ventral cell wall lining. (17) Entire cell platform moved upward to meet cell rim and form cell cap. (18) Basoventral section of cell cap indented to meet provisions that change shape because of this contact. (l, lower surface of cell provisions; m, Dufour's glandular droplets deposited adjacent to egg; n, point of attachment of anterior section of platform to ventral cell wall lining; o, dorsal surface of egg; p, lateral thickness of cell cap after it is adjoined to cell rim; q, the bent section of cell cap caused by activities of female, resulting in its inner surface contacting the provisions that quickly causes the shape of cell provisions to change).

action. (*Hylaeus* employs a similar behavioral pattern to accomplish the same objectives [Torchio 1984].)

The female then exited the cell, turned a half-somersault, returned to the cell, added more nectar to the provisions, backed out of the cell, turned another half-somersault, backed into the cell, and repeated her abdominal tamping of the cell provisions. This was followed by preening activities. The female then repeated the abdominal sweeping and tamping activities until 2045 hours immediately before egg desposition. At that time, the upper section (half) of the provision was 2 mm thick, and its surface was angled only a few degrees from vertical. The lower section of the provisions was 3-4 mm thick, and its surface was angled at 35° from vertical (Fig. 14).

Egg Deposition

At the end of the provisioning period, the female was positioned with her thoracic terga resting on the posterior section of the cell's platform, her head faced the nest entrance, the abdomen was slightly arched upward, and all legs were pressed against the cell wall. She began to pump her abdomen rapidly for 1 min, and an opening appeared at the

terminus of her abdomen. The diameter of this opening increased each time the sting was partially protruded from that orifice. The rapid pumping ceased, the sting was fully protruded and deflexed to a position nearly perpendicular to the posterior abdominal terga, and the tip of the abdomen was opened to its widest limits. The female then began a slow, heavy pumping motion of her abdomen and, almost immediately, the egg began to extrude from the oviduct opening. The egg advanced a short distance through the oviduct opening with each successive pumping motion of the abdomen; it was almost fully extruded from the posterior tip of the abdomen just 8 s after the heavy pumping motion of the abdomen began. At that point, the posterior tip of the curved egg (first to emerge from the oviduct opening) was touching the cell provisions, and its anterior tip remained attached to the posterior tip of the bee's abdomen.

The female stopped all bodily movements for 5 s as a clear liquid accumulated around the anterior tip of the egg attached to the abdominal opening. Then she suddenly jerked her abdomen upward until the anal opening touched the ceiling (upper wall) of the horizontal cell 1.5 mm forward of the cell provisions. She quickly lowered her abdomen to a horizontal position, but the rounded anterior

Fig. 19–23. Egg and embryo development of *C. kincaidii*. (19) Egg with attached (anterior) tip flattened as surrounding fluid dries. (20) Egg during mid embryogenesis as chorion softens and outline of developing head capsule is viewable. (21) Early stage of eclosion with chorion (stippled) split across the four anterior spiracles of the first instar. (22) Embryo-larva with posterior tip of chorion attached to upper cell wall. (23) First instar in linear position on upper cell wall and covered by egg chorion (stippled) across head, venter, and last two body segments. (r, egg chorion (stippled) covering first instar larva).

nathous position and began biting at the lower base of the cell cap. At the same time, she initiated a walking motion with all legs, and she pressed the tip of her abdomen to the lower surface of the glass tube to better anchor her body. These biting and walking motion activities continued for 20 min, whereupon the female abruptly crawled backward a short distance within the glass tube, turned a half-somersault, crawled back to the cell, and rapidly smeared streaks of clear anal liquid on the outer surface of the cell cap as previously described. She then reversed her position in the tube, returned to the cell, and continued the chewing-walking activities at the base of the cell cap for an additional 20 min. These activities continued until 2156 hours, during which time two additional depositions of anal fluid were applied to the cell cap.

Each application of anal liquid added to the outer surface of the cell cap caused the entire structure to soften temporarily (15–20 min). As a consequence, the pushing action resulting from the in-place walking motions, combined with the mandibular chewing actions of the female working at the base of the cell cap, caused that section of softened cell cap to gradually bend inward until its inner surface touched the cell provisions (Fig. 18). When that occurred, the additional contact area on the cell provisions allowed the liquefied pollen-nectar stores to flow upward along the angled, inner surface of the cell cap (Fig. 18) until

surface tension pressure was stabilized on the liquefied provisions. As a result, the angulation of the lower cell provisions moved to near horizontal, and its surface became cup-shaped because of changed surface tension pressure. At the same time, the thickness of cell provisions attached to the upper cell was reduced by as much as 1 mm.

This indirect method of reshaping cell provisions proved to be an important final step in the preparation of the cell for the development of immature forms. Reduction of cell provision thickness along the upper cell wall increases the distance between egg attachment and cell provisions. This helps to assure sufficient space for movement and attachment of the embryo-larva to the upper cell wall preparatory to the larva's movement onto the surface of the cell provisions. The cup-shaped surface formed on the lower cell provisions subsequently supports the floating, C-shaped developing larva after it migrates from the upper cell wall (described below).

Because of these combined activities, the original cell platform was physically reshaped into the cell cap when its ASP was moved upward until it was bonded to the cell rim. Subsequently, the basal section of the cell cap was indented until it touched the cell provisions. The contact of the cell cap with the cell provisions changed the surface tension of this liquefied mass. In turn, this caused the provisions to flow until it was reformed in a specific

manner (Fig. 18) that accommodated the successful development of immature stages as they floated on surface of the cell provisions. The CP therefore plays an important and varied role in the construction of *Colletes* cells.

Late Embryogenesis

The following descriptions of embryogenesis and egg hatching are based on composite in vivo observations of six eggs each immersed in paraffin oil 2 or 3 d after they were deposited in cells constructed in glass tube nests; 17 additional eggs also were observed in situ. Results indicate that in vivo methods do not adversely affect embryological development and egg eclosion.

Orientation of the embryo has been established based on direct observations made during the deposition of 13 eggs laid in glass tube nests. These results are the same as those obtained in previous studies (Torchio 1984, 1986; Torchio & Trostle 1986). These observations suggest that eggs of Hymenoptera are emitted posterior-end first (Apocrita) through the oviduct opening (Apoidea, most Aculeata) or through the ovipositor (Parasitica [Krombein et al. 1979] and *Sapyga* [Torchio 1972]). Also, the dorsal-ventral surfaces of the egg are directly associated with the same surfaces of the bee's oviduct. As a consequence, the longer curved surface of a *C. kincaidii* egg is its dorsum (Fig. 16, arrow o), and the anterior tip is attached to the cell wall (Fig. 18 and 19).

The head and 13 body segments of the embryo are clearly distinguishable 90–96 h after the egg is deposited. The mandibular and two maxillary head segments are represented by three pairs of prominent knobs on the ventral surface of the head capsule, and both tips of the embryonic membrane (serosa) have separated from the vitelline membrane. This is early stage 9 as described by DuPraw (1967) for the honey bee. As stage 9 progresses, the mandibles, maxillae, and labium develop into discernible structures, and the embryo slowly increases in size.

After 3–4 h of continuous growth, the embryo's head and anal segment touch the embryonic membrane, and the head and mouthparts move feebly (the initiation of muscle contractions signals the beginning of stage 10). The frequency of these head and body movements slowly increases during the next 10 h as the embryo consumes fluids and increases in size. At the end of this time, the embryo has grown so that it nearly fills the inner space of the egg, and the embryonic membrane is stretched taut across the front of the head capsule and around the anal segment. The hexagonal reticulations that adorn the outer surfaces of both tips of embryonic membranes of other bee taxa (Torchio 1984; Torchio & Trostle 1986) are represented in *C. kincaidii* by narrowly separated, round bumplike structures that cover only the anterior and anterolateral sections of the embryonic membrane.

The tracheal system becomes visible as it is filled with metabolic gas within 30 min after the embryonic membrane is stretched taut around the embryo (this occurs in stage 9 of the honey bee embryo [DuPraw 1967] and other bee taxa [Torchio & Trostle 1986]). The venter of the fully developed embryo, as in other Apocrita, faces the dorsum of the egg, and its head faces the anterior (attached) tip of the egg (prerotated embryo).

Ten minutes after the tracheal system fills with gas, the embryo begins a slow, passive 180° rotation on its long axis within the stationary embryonic membrane. The embryo rotates for 6 h with 20–40-min pauses at 90° and 120°. The embryo periodically moves its mandibles and anal segments feebly during each pause, but the frequency of these movements increases after the embryo completes its 180° rotation. During the next 30 min, the embryonic membrane softens, splits open, and rapidly disintegrates.

Egg Eclosion

The anterior fourth of the egg chorion begins to soften soon after the dissolution of embryonic membrane is completed. This softening allows the chorion to slowly collapse around the embryo's head capsule (Fig. 20) during a 2-h period as the embryo begins to undulate its foregut. (We presume that undulations of the foregut combined with the feeble movements of the mouthparts allows the embryo to imbibe fluids through the buccal cavity.) The posterior section of egg chorion also softens as body segments 2–5 slowly enlarge dorsad of each line of spiracles during an 8-h period. As this swelling (or ballooning) continues, the ventrolateral and ventral tubercles also enlarge, and body segments 6–13 are slowly uplifted until they are nearly horizontal and parallel to the upper cell wall (Fig. 21). Immediately thereafter, a narrow split in the egg chorion appears across each ventrolateral tubercle on body segment 3 of the embryo. Each split rapidly lengthens to expose the ventrolateral tubercles on body segments 2–4 within a 3-min period. The split then widens dorsally and posteriorly during the following 3-min period until spiracles on body segments 2–5 are fully exposed (Fig. 21).

The embryo-larva continues to swell at a slower rate for an additional 2 h, during which time the posterior body segments are lowered until they are positioned at a 45° angle below horizontal. At the same time, the swelling process forces the split in egg chorion to extend posteriorly until spiracles on body segments 6–8 are exposed. Near the end of this 2-h period, the embryo again arches its posterior body segments upward in a slow swinging motion for 3-min until its posterior tip attaches to the upper cell wall (Fig. 22). This swinging motion is always interrupted by two or three pauses of 15–30 s each.

During egg deposition, the female normally attaches the anterior tip of the egg to the cell wall

several millimeters in front of the provisions. Subsequently, the posterior tip of the egg is raised by the embryo-larva until it too is attached to the upper cell wall immediately in front of the cell provisions (Fig. 22). However, the female sometimes attaches the anterior tip of the egg nearer the surface of cell provisions; as a result, the posterior body segments of the embryo-larva are immersed in cell provisions when these segments are lifted.

First Instar. Immediately after the anal segment is attached to the cell wall, the larva initiates vigorous, continuous movements of its mouthparts as it actively swallows additional embryonic fluids. In addition, its head moves freely in all directions and, for the first time, it moves individual body segments independently. These newly acquired larval movements suggest that the active process of hatching ends when the posterior tip of the *C. kincaidii* embryo-larva is attached to the cell wall, even though the egg chorion continues to loosely cover much of the first-instar larva.

During the next 8-h period, the C-shaped first instar swallows the remaining embryonic fluids (and possibly air), but it remains attached to the cell wall lining only by its anterior and posterior tips. As these materials are consumed, the larva swells slightly, which allows the split chorion to extend posteriorly to the 11th body segment, thus exposing the remaining spiracles.

Soon after all body spiracles are exposed, the larva moves from its C-shaped form to a linear, horizontal position within the egg chorion (Fig. 23) during a 2-h period by means of peristalsis. Peristaltic waves move posteriorly and then anteriorly across all body segments in an alternating sequence. These movements force the head and anal segment to slowly slide in opposite directions within the pliable egg chorion, thereby allowing the larva to gradually straighten into a linear position. As this occurs, the egg chorion covering the body sternum immediately behind the head (body segment 1) and directly in front of the anal segment (body segment 12) touches the cell wall first, followed by contact of body segments 2 and 11, 3 and 10, 4 and 9, 5 and 8, and 6 and 7.

The now-linear larva continues peristalsis for an additional 2 h, but these movements are changed in two distinctive ways—peristaltic waves move only posteriorly, and muscle contractions are greater on one side of each and-all body segments (versus equal muscle contractions across the length of each body segment during the previous 8-h period of peristalsis). These changes (factors) allow the entire larva to slide posteriad a short distance within the egg chorion (factor 1) as the posterior body segments slowly twist (factor 2) and angle off to one side.

The egg chorion continues dissolving throughout this period, but apparently molting fluid is not equally distributed across the endochorion. As a consequence, the head capsule and the last two

body segments remain encased by egg chorion, and these coverings are interconnected ventrally by a narrow band of chorion residue attached to the upper cell wall (Fig. 23). As the larva moves posteriorly via peristalsis, the head capsule is pulled in the same direction for a short distance until much of the labial surface contacts the inner surface of egg chorion. As a result, the head stops its posterior movement, but continued peristaltic movements by the larva creates a pulling force on the stationary head capsule which, in turn, causes the apical section of the capsule to lift slowly upward until the mouthparts are moved from a hypognathus to a prognathus position (Fig. 24).

First Larval Molt. Soon after the head capsule is tilted upward, the larva enters a 5- to 6-h "rest" period, during which time head and body movements nearly cease. This period of rest is suddenly terminated when the larva lifts its last two body segments from their attachment to the cell wall and resumes continuous head and mouthpart movements associated with swallowing. The last two body segments, once freed of the substrate, are partially telescoped into the 10th and 11th body segments before the anal segment is reanchored to the cell wall a short distance anterior of its previous point of attachment. These specific activities initiate continuous peristaltic movements that originate near the posterior tip of the larva and end at the posterior margin of the head capsule (which remains pressed to the cell wall).

As peristalsis continues, the entire larva moves forward a short distance within the partial egg chorion covering as the three anterior body segments again swell dorsally for 14–15 min. The combination of forward thrusts by the larva (peristalsis), the ballooning of anterior body segments, and the ventral surface of the head firmly appressed against the cell wall work in concert to force the head capsule to be tilted forward onto its labrum. As the anterior body segments continue to swell and move forward, the head capsule continues to tilt under the body until it comes to rest with its mandibles touching the ventral surfaces of the first two body segments (Fig. 25).

Within 1 min after the head capsule is fully tilted under its body (vertex pressed to endochorion attached to upper cell wall), a short longitudinal split in the first-instar cuticle appears immediately above each spiracle of body segment 2. These cuticular splits expand rapidly and extend across the dorsum of that segment until they coalesce near the posterior margin of the first body segment. The cuticle then continues to split posteriorly to body segment 11 along both spiracular lines. As this occurs, the dorsum of first-instar cuticle continues to split anteriorly across body segment 1 and through the dorsomedial area (vertex) of the head capsule. At the same time, the cuticular exuvium of the first instar that covers the remaining dorsal surface of the second instar slides posteriorly as a result of peristalsis. That section of split cuticle below the

Fig. 24 and 25. Egg eclosion by second instar *C. kincaidii*. (24) First instar with head lifted and mouthparts in a prognathus position. (25) Early phase of molting from first to second instar (dark stippled area = exuvium of first instar covered by egg chorion; light stippled area = exuvium of first instar only). (s, tracheal remnants of first instar pulled from spiracular openings of second instar because of attachment to egg chorion—first-instar exuvium (stippled) sliding posteroventrally during eclosion).

spiracular line has narrow ribbons of undissolved cuticle attached to the spiracular collars of the first-instar exuvium. As this lower section of cuticle slides posteroventrally, the spiracular exuviae and tracheal remnants of the first instar are pulled from the spiracular openings of the second instar and they are also carried posteroventrally (Fig. 25, arrow s).

Peristalsis is discontinued, the larva lifts its head away from the cell wall toward a vertical position and this movement forces the entire head capsule to slide free from its encasement in the egg chorion—first-instar exuvium. Subsequently the last two body segments are lifted from the substrate and repeatedly telescoped as they also slide free from the last vestige of egg chorion covering. Eclosion is now completed.

Second Instar

The expanded anterior body segments of the fully eclosed larva deflate soon after the spiracular openings are cleared of tracheal remnants of the previous instar. As a result, the head returns to a hypognathus position and peristalsis is resumed. Peristalsis allows the larva to slide posteriorly across the attached residue of egg chorion and first-instar exuvium as it turns onto its side to float and feed on the cell provisions. To accomplish this, the larva lifts its entire head capsule free from its attachment to the cell wall and telescopes its head into the first body segment before the head capsule is lowered and reattached to the cell wall posteriad of the previous point of attachment. This forces the first body segment to greatly expand until it lifts from its attachment to the cell wall. As that occurs, a peristaltic wave travels segmentally the length of the body until it reaches the posterior tip of the larva. At this point, the last two body segments are lifted from their attachment to the substrate, distended to their maximum length posteriorly, and angled off to one side before the anal segment is reattached to the substrate. The larva then moves posteriorly a short distance as a peristaltic wave motion is directed anteriorly. These repetitive peri-

staltic movements allow the larva to travel backward with some speed; at the same time, its body begins to tilt onto one side in response to each reattachment of the anal segment off to one side.

The larva thus backs onto its cell provisions by simply continuing these peristaltic movements. The surface tension of liquefied cell provisions is sufficiently strong to support the weight of the larva when that individual first places its anal segment onto the surface of provisions as it angles posteriorly. As a result, the larva continues to tilt onto its side as it moves on the cell provisions (Fig. 26) until it assumes a C-shaped position. Once the larva is turned onto its side, it discontinues peristalsis and slides downward across the surface of the steeply angled liquefied provisions (Fig. 27) until it reaches the subhorizontal, cup-shaped surface of those provisions (Fig. 28) 1 h later. The larva's passive movement from the top edge of the provisions (in a horizontal cell) to a central position on the nearly horizontal surface of those provisions occurs as a result of a combination of factors, including the force of gravity, the cup-shaped surface of the liquefied cell provisions (caused by strong surface tension forces) that prevents the larva from floating to the outer edges of the provisions, the decreased angulation across the lower sections of the cell provisions that reduces gravitational pulling forces, and the strong C-shaped form of the larva that increases flotation potential.

The feeding behavior of this and subsequent stadia is nearly identical to that of *Hylaeus bisinuatus* Forster described elsewhere (Torchio 1984). This repetitious feeding pattern begins when the larva straightens its anterior body segments and opens and closes its mandibles as the head and anterior body segments are returned to their original position. These movements force liquefied provisions into the mouth of the larva, and at the same time the larva moves a short distance anteriorly but in the same angled direction as that of its curved body. As a result, this and subsequent instars move in a circular pattern within a central area on the surface of cell provisions until the provisions are largely consumed.

Fig. 26–30. Immature development and nest cap of *C. kincaidii*. (26) Second instar backing onto surface of cell provisions. (27) Second instar sliding down angled surface of provisions. (28) Second instar floating on horizontal, cup shaped surface of provisions. (29) Predefecating fifth instar larva. (30) Nest cap in glass tube nests.

Unlike *H. bisinuatus*, the feeding larva of *C. kincaidii* (second through fifth instar) frequently pulls its elongated anal segment into the penultimate body segment, during which time quantities of cell provisions flow into the anus and fill the rectal cavity. The feeding larva then carries its head to the posterior tip of its body, where it spreads its mandibles and inserts the posterior tip of the body well into the mouth cavity. As the gnathal segments close around the inserted body segments, the anal segment is rapidly pushed outwardly from within the penultimate body segment, forcing liquefied provisions to flow from the rectal cavity directly into the buccal cavity. Large quantities of cell provisions are consumed by this unique method. It is possible that glandular secretions are added to those cell provisions pulled into the rectal cavity.

Subsequent Larval Molts

Molting by the second, third, and fourth instars follows an identical sequence initiated when the larva abruptly stops feeding, followed by 2–3 min of inactivity as spiracles change from nonreflective to highly reflective structures. Muscles attached to the spiracles are flexed repeatedly and rapidly as the anterior body segments again swell throughout an 8-min period. The angulation of the larva's C-shaped form increases as the body swells and the head capsule is gradually distended.

Shortly after the pumping motion of the spiracles is discontinued, a narrow split appears along each midspiracular line across body segments 2–5. The spiracular exuviae and tracheal remnants of (for example) the second instar remain attached to the ventral margin of the split cuticle that subsequently slides posteroventrally. As this occurs, the tracheal remnants of the second instar are pulled through the spiracular openings of the developing third instar. The lateral split of the second instar cuticle continues posteriorly to body segment 11 and extends anterodorsally to the dorsal midline of body segment 2. The split continues anteriorly across the length of body segment 1 and through the vertex of the head capsule, where it ends at the upper margin of the clypeus. The larva resumes peristalsis, which is shortly followed by an elongation of the entire body and a rapid deflation of the anterior body segments. The head capsule of the third instar is withdrawn from its encasement within the split second instar head capsule exuvium by a lifting and tilting of the head upward.

When the head of the third instar is lifted free of its encasement, the ectodermal exuvium that lined the mouth of the second instar is pulled from the mouth opening of the third instar in the form of a soft, transparent strand. As peristalsis continues, the dorsal and ventral remnants of the second instar exuvium slide posteriorly. At the same time, the third instar lifts its anal segment from its at-

tachment to cell provisions until that segment slides free from its encasement in the second-instar exuvium. As this occurs, a long strand of clear material (two-thirds the length of the second instar and representing the exuvium of the second-instar hind gut lining) is also pulled from the anus of the third instar. Soon afterward, the entire second-instar exuvium floats free from the posterior tip of the third instar and, as in *H. bisinuatus* (Torchio 1984), continues to dissolve after its disassociation from the larva.

Molting from first to second instar was completed in 1 h, whereas molting of each subsequent instar required 20–25 min. Also, molting from first to second instar occurred within two layers of exuvial coverings (egg chorion, in part, and first-instar exuvium) as the linear larva remained attached to the cell wall. The second and subsequent instars retained a C-shaped position as they floated on cell provisions throughout molting periods. Yet the actual sequence of molting was similar among all instars. In fact, some actions described for second and subsequent instar molts that were not included in the descriptive account of the first-instar molt (for example, exuvial strands pulled from the mouth and anus) may have actually been hidden from view by the wrinkled egg chorion residue that covered both the head and posterior body segments of the first instar.

Fifth Instar

Development. Because of time constraints, observations of the fifth instar were limited to frequent spot checks. As a consequence, the following account is based on a composite of several, short-term observations made in the field, laboratory, and greenhouse.

Within 15 min after the larva completed its molt into a fifth instar, it began to consume provisions as described (including transfer of provisions from the rectal cavity directly into the buccal cavity). At this time, less than one-quarter of the provision was consumed, and its shape was not changed. However, during the next 72 h, the larva consumed the remaining provisions as it grew rapidly and its curved body encircled the basal circumference of the cell. The larva then moved into a more linear form preparatory to depositing fecal particles (Fig. 29).

As provisions were consumed by the fifth instar, the cell fragrance changed from sweet nectar to a sour, pungent odor. The intensity of this foul odor increased during defecation and was retained in the cell until emergence occurred the following year. When a cell containing a live prepupa was opened in mid-winter, this extremely foul odor immediately filled the laboratory (3.6 m² by 2.4 m) and was detectable 6 h later.

Defecation. Fecal deposition was initiated within 24 h after the provisions were consumed, when the larva began discharging a continuous strand of

feces. This soft, wet material was brushed onto the inner surfaces of the cell lining as the larva moved its extended terminal body segment back and forth across those surfaces. Periodically, the larva stopped defecating, moved a short distance (by peristalsis), and resumed discharging fecal material. During this 72-h period, the larva moved 360° through the long axis of the cell until its head returned to the predefecating position and faced the cell cap. At that time, all or most of the inner cell surface was coated with feces, which continued to dry and darken for several weeks after its deposition. Near the end of the defecation period, the anal segment became less extended, and feces were subsequently deposited as individual particles interconnected by hardened, brown, translucent strands. If the larva happened to move across these interconnected particles before they dried, the interconnecting strands were broken and the individual integrity of each fecal particle was lost and melded into the surrounding fecal smear. Sometimes, these individual fecal particles became attached to the larva as it passed over them and they remained attached to the larva throughout the wintering period.

Soon after defecation was completed, the larva transformed into a prepupa by first moving into a strongly C-shaped form with the vertex of its head facing the cell cap. The larva then turned opaque white; the body segments became clearly defined; all movement stopped; and the body softened somewhat 4–6 d after defecation was completed. The larva overwintered as a slightly flaccid prepupa.

The methods by which *C. kincaidii* deposited fecal material were similar to those described for *Colletes ciliatoides* Stephen (Torchio 1965). However, the *C. ciliatoides* larva completed a second 360° turn through the long axis of the cell to deposit a clear varnish material over the fecal layer. This material (Malpighian excreta) was also deposited anally, and it quickly dried into a hardened, reflective layering that separated the larva from its feces. Claude-Joseph (1926) noted that the larva of *Colletes araucariae* Friese secretes a yellowish fluid from the mouth that forms a secondary envelope on the inner cell walls. Michener & Lange (1957) describe a cocoonlike layer that covers the larval feces in cells of *Colletes michenerianus* Moure. Apparently, *Colletes* secretes species-specific materials onto larval fecal layers.

Pupa

Four pupae obtained from the field in mid-March 1985 were reared at 26°C and observed daily. The following descriptions are based on observed coloring sequences of the developing adult under the pupal cuticle: Days 1–5, pupa entirely white. Day 6, setal pores, compound eyes, and ocelli begin to color. Day 7, hairs on distal section of abdominal tergites darkening; compound eyes and ocelli darker; tarsal claws, genital capsule, mandibles, and

posterior abdominal segments beginning to color (reddish). Day 8, most abdominal tergites colored except anterior tergites 1–2 dull white with circle of darkened hair on each side of midline; pronotum and scutum coloring. Days 9–10, no observations. Day 11, head and thorax dark brown; basal antennal segments brown, flagellar segments with red tinge; femora and tibiae dark brown, tarsal segments with red tinge; first two abdominal tergites and most abdominal sternites rapidly coloring. Day 12, pupal cuticle softens; head capsule and thorax black; proboscis and tarsal segments brown; abdomen mostly colored except anterior tergite; abdomen and legs move periodically; clear liquid exuding from lateral areas of intersegmental lines on posterior abdominal segments. Day 13, pupal cuticle continues to soften; pupa entirely colored; additional liquid exuded from intersegmental lines on abdomen; entire pupa capable of movement; genital capsule pulled inside body cavity. Day 14, cuticle completely collapsed around adult; no additional coloring noted; no additional liquid exuded from intersegmental lines on abdomen. Days 15–16, pupal skin is shed and adult stage begins (average pupal period, 16 d; $n = 4$).

Nest Associates

***Epeolus compactus* Cresson.** This cleptoparasitic bee was found patrolling and entering nests of *C. kincaidii* during the active nesting period of the host species. Eggs and larvae of the parasite were recovered from host cells during field excavations, and those specimens were subsequently reared in the laboratory. Those observations have been reported elsewhere (Torchio & Burdick 1988).

***Paravilla fumosa* Hall.** Adults of this bombyliid fly were also collected at the nesting site of *C. kincaidii* during peak nesting activities. Immature forms were recovered from several host cells when active host nests were excavated (July 1985), and a number of mature larvae were subsequently reared to adults. Other *Paravilla* species are known to attack ground-nesting bees (Jack Hall, personal communication), but this represents the first host record for *P. fumosa*.

Fungi. An undetermined fungus was found in all old, vacated cells uncovered during field excavations. However, no fungus was found in any live or dead cells constructed in the field or greenhouse during the tenure of these studies (1985–86).

Cells, immatures, and adult material of *C. kincaidii*, *E. compactus*, *P. fumosa* and fungi have been deposited in the USDA-ARS Collection, Bee Biology and Systematics Laboratory, Logan, Utah.

Discussion

Dufour (1835) discovered a large abdominal gland (subsequently named the Dufour's gland) in some bees, and he suggested that cell linings produced by *Colletes* and related taxa may be derived

from the contents of this gland. Nearly a century passed before Ferton (1923), Claude-Joseph (1926), Janvier (1933), Rayment (1935), and Skaife (1950) each observed cell linings of colletid bees (*Hylaeus*, *Policana*, *Colletes*, *Diphaglossa*, *Euryglossa*) deposited as a rapidly drying oral secretion that was brushed onto the substrate with the glossa. Therefore, it was assumed that the cell membrane produced by colletid bees was derived exclusively from a salivary origin.

Semichon (1906) pioneered studies in associating cell lining materials with Dufour's gland secretions based on solubility and melting-point comparisons. Malyshev (1936) later found that apoid cell linings varied from one taxon to another in melting-point temperatures and solubility in various compounds. These studies were expanded in the 1960s until the cell linings of most of the higher bee taxa were analyzed. Batra's (1972) review of the literature included results of melting points, solubilities, and infrared spectral analyses on cell linings of three species of bees. In that paper (p. 215) she concludes, "The most primitive family of bees, the Colletidae, evidently secretes a silken membrane from the mandibular glands. Although large Dufour's glands and integumental glands are present, there is no evidence for their use in nest making and they may produce pheromones."

The solubility studies predicted that the chemistry of apoid cell linings was complex and somewhat diversified. May (1974) confirmed the complex features of cell-lining chemistry in her analyses of cells constructed by the halictine bee, *Augochlora pura* (Say). Bergström (1974) identified macrocyclic lactones in the Dufour's gland of *Colletes* and several halictine bees. Hefetz et al. (1979) demonstrated that the cell linings of *Colletes* contain a polyester constructed of corresponding lactones found in the Dufour's gland of the female bee. Albans et al. (1980) studied the Dufour's gland secretion of several European *Colletes* species and independently arrived at the same conclusions reached by Hefetz et al. (1979). Batra (1980) observed Dufour's gland secretion transferred from the sting to the glossa each time a *Colletes inaequalis* Say female somersaulted in the nest as cell lining materials were deposited. Based on that evidence, she concluded that Dufour's gland secretion was imbibed during sting-glossa transfers and regurgitated as the glossa brushed across the cell wall. McGinley (1980) used this information to explain particular glossal features of colletid bees. Cane (1983) followed with a chemocladistic analysis of those bee taxa having lactone-producing Dufour's glands (Colletidae, Halictidae, Oxaeidae). The combined results of these studies pointed to an obvious but somewhat incomplete presumption—that *Colletes* produces its entire cell lining from Dufour's gland secretion rather than from a mixture of salivary and Dufour's gland secretions.

Our direct observations of the entire cell-constructing process by *C. kincaidii* suggest that each

of the conclusions reached in previous studies dealing with apoid cell linings are partially correct. Our observations, for example, now demonstrate that *Colletes* uses both cephalic and abdominal gland materials to construct its cell lining and nest plug (Fig. 30). The (presumable) salivary liquid is applied sparingly but during long periods of time, whereas the (presumable) Dufour's gland liquid is deposited in larger quantities but infrequently. As a result, equal or nearly equal quantities of these two gland materials are used to construct the *Colletes* cell lining. The quantitative differences in amounts of glandular material secreted over time, therefore, requires little storage capacity of salivary glands and maximum storage capacity in the Dufour's gland. The large Dufour's gland of *Colletes* may indicate its high storage capacity rather than its singular importance in producing the cell lining of *Colletes* bees.

Our observations also have demonstrated that salivary liquid was always brushed over any surface of the cell before the deposition of Dufour's gland liquid on that same surface. This sequential layering of glandular materials also occurred when *C. kincaidii* transferred small quantities of presumed Dufour's gland liquid from the sting or anal orifice (when sting not exerted) to the glossa accompanying each half-somersault. In fact, somersaulting usually followed extended periods of salivary deposition when those liquids began solidifying directly on the mouthparts before they were deposited on the nest wall. Therefore, the primary purpose of somersaulting was to add Dufour's gland material to the glossa. The transferred Dufour's gland liquid then acted as a solvent on salivary secretions that dried more slowly as the female continued brushing surfaces previously coated.

The purposeful layering of glandular secretion strongly suggests that salivary material deposited by *Colletes* carries enzymes that open and then cross-esterify the lactones produced in the Dufour's gland into the polyester layers found in *Colletes* cell linings. These enzymes apparently remain active well after salivary liquids harden and, as a consequence, Dufour's gland liquids solidify only after contact with the salivary coating.

Albans et al. (1980) demonstrated that the contents of *Colletes* Dufour's glands failed to dry when applied directly to a glass plate, and they predicted that polymerization of the Dufour's gland secretion may be mediated by an enzyme probably derived from salivary glands. The discovery of pollen grains embedded in the polyester lining was used by Albans et al. (1980) to support their hypothesis that pollen grains embedded in *Colletes* cell linings are carried from the bee's mouth when salivary gland materials are secreted. In our study, however, we observed that residue pollen grains became embedded in the lower cell wall lining when the nesting female periodically brushed additional salivary liquids across those surfaces during pollen-nectar provisioning periods.

Flower & Kenchington (1967) demonstrated how different sections on the salivarium in larval apoids (*Apis*, *Bombus*) and *Vespa* produce different materials that are combined enzymatically into the silklike material that is subsequently secreted through the salivary lips. Therefore, adult *Colletes* may produce polyester cell linings by using similar pathways through which Apocrita larvae produce cocoon silk. A chemical analysis of oral secretions produced by *Colletes* should now be undertaken to determine if polymerization of lactones occurs through enzymatic catalysis or through an acid-base catalysis. It would then be interesting to analyze the chemistry of *Hylaeus* cell linings (produced only from salivary material [Torchio 1984]) to determine if similar salivary components are present in the cell linings of both colletid genera.

After cells of *C. kincaidii* are sealed, the waterproof and extremely hygrophobic cell linings serve to protect cell contents from flooding, desiccation, spoilage, etc. Inside these sealed cells, however, some Dufour's gland secretions are retained as a liquid; it slowly solidifies during immature development (droplets deposited adjacent to egg and liquid coating the attached, anterior tip of egg). The solidification of Dufour's gland secretions indicates that biosynthesis of polyesters continues after cells are sealed. The major byproduct of this biochemical reaction (lactone to hygrophobic polyester) is water. Thus, egg attachment to the cell wall is increased as Dufour's gland liquid covering the anterior tip of the egg solidifies, during which time water is slowly released into the air space of a waterproof cell having liquefied provisions. Because all liquids disappear from within the cell as the prepupa enters the wintering period, we assume that available liquids are slowly absorbed by the developing larva and are retained in body tissues of the prepupa through the winter. May (1972) measured unaccountable weight gains of more than 60% in developing *Augochlora pura* larvae, which she attributed to water uptake. Although the functional bases for water uptake by larvae of some bee taxa are not currently understood, the complicated methods used by *Colletes* to increase the water content in waterproof cells (including liquid secretions by the pupa) suggests that water plays an important role in assuring the successful development of immature forms.

Results obtained from the weight studies showed that each series of cells was constructed in an identical pattern irrespective of the medium in which the cell series was constructed (glass tubes or field nests) or the pollen host species used to provision one or all cells in a series. By rearing this material, we were able to confirm the following: Females are produced in the first constructed cells of each series, and males are found in the outer cells of the same series. Average prepupal weights per cell vary from 15 to 20% between cell series, but the first constructed cell of each series contains the heaviest larva with succeeding cells (either sex) each con-

taining a lighter prepupa. We also learned that weight loss from prepupa to pupa was 1–1.8% for males and 2.2% for females ($n = 15$ per sex), and weight loss from pupa to adult was 5.5–13% for males and 9–11% for females ($n = 15$ per sex). The minimum weight loss from prepupa to pupa may be associated with water retention in the body tissue of the larva, and the increased weight loss from pupa to adult may be associated with the release of quantities of liquid by the pupa during its coloring phase.

The embryology of bees, as well as eclosion from the egg and molting between instars, is known primarily for *Apis* (Nelson 1915; DuPraw 1961, 1967). Recently, however, Torchio (1984, 1986), Torchio & Trostle (1986), and Trostle & Torchio (1986) have observed (in vivo) late embryogenesis and immature development of several additional bee species and a masarid wasp. One of these papers (Torchio 1984) involved a similar greenhouse study on the in-nest biology of another colletid bee, *Hylaeus bisinuatus* Forster. It seems worthwhile, therefore, to compare *Hylaeus* with *Colletes* by including recently discovered characteristics (Torchio 1984, and various features described above) to baseline information available in the literature. Although these comparisons are limited to two genera placed in a large family of bees, they may prove useful in establishing a broadened foundation on which to develop future biosystematic studies of the Colletidae.

Some of the traditional characteristics currently used to distinguish *Hylaeus* from *Colletes* are listed in the following outline: *Hylaeus* females are without scopae (pollen-nectar resources carried in crop); the body is nearly hairless and colored black, or it is black but interrupted with some yellow markings, or the entire abdomen is reddish. Females nest only in existing holes. The front wings have two submarginal cells; the hind wings with vein m-cu straight, not arcuate. Alternatively, *Colletes* females have a well developed scopa on each hind leg; the body is covered with a dense hair pile; the thorax and legs are not maculated; the front wings have three submarginal cells; the hind wings have the posterior portion of vein m-cu arcuate outwardly; and nests are usually excavated in soil.

Some recently discovered characteristics also distinguish these two genera: The embryo of *Hylaeus* rotates 90° on its long axis; the first instar ecloses completely from egg chorion and feeds on cell provisions; the fourth instar consumes all of the remaining cell provisions; the fifth instar is a non-feeding form; the pupa has only a few spines and tubercles, and it does not release visible liquids; in addition, the cell lining is spun only with oral (salivary) secretion. Conversely, the embryo of *Colletes* rotates 180°; its second instar ecloses from the egg chorion, and the first instar feeds exclusively on embryonic fluids; the fifth instar consumes the remaining cell provisions before defecation. The pupa has many more spines and tubercles (Torchio

& Burwell 1987), and it releases quantities of clear liquids prior to molting. In addition, the cell linings of *Colletes* are composed of equal quantities of cephalic and abdominal gland materials.

Those characteristics listed above as “recently discovered” have not been studied in other colletid bees. It is premature, therefore, to include them in a cladistic analysis of the family. However, a deductive comparison of these recently discovered characteristics is justified when only these two genera are considered and comparable information is available within a legitimate out-group. For example, the embryos of known Apocrita wasps (Trostle & Torchio 1986) and bees (except *Hylaeus*) rotate 180° on their long axes prior to eclosion. Therefore, the 90° rotation by *Hylaeus* appears to be a derived characteristic.

Conversely, *Hylaeus* follows the majority rule in that the first instar ecloses from the egg before it feeds on cell provisions. *Colletes* obligatorily consumes embryonic fluids as a partially hatched first instar and completes eclosion from the egg chorion as a second instar before feeding on cell provisions. Three additional bees (*Xylocopa uclestensis* Perez, *Osmia latrillei* Spinola, *Hoplitis tridentata* (Dufour and Perris)) also complete eclosion from the egg chorion obligatorily as second instars (unpublished data), and at least two other species (*Osmia californica* Cresson, *O. montana* Cresson) facultatively complete eclosion from the egg as first (or commonly) as second instars (unpublished data). These examples suggest that the first- or second-instar eclosion habits have arisen independently in several families of bees. However, there is an apparent functional relationship between species that complete eclosion as first or second instars; i.e., the volume of the egg is large relative to the size of the first instar in species that complete eclosion from the egg chorion as second instars. Because all embryonic fluids are consumed by all known bee larvae before eclosion from the egg chorion, egg size or the volume of enriched embryonic fluids surrounding the embryo larva, or both, may be the most important factors that determine which instar ecloses from the egg. This variable characteristic is probably widespread in Apoidea, because egg size varies dramatically among many taxa and even between species groups in the same genus (*Osmia*).

The last instar larva of all known bees and masarid wasps (except *Hylaeus*) consume cell provisions. Thus, the unique feature of a nonfeeding fifth instar is probably a derived characteristic of *Hylaeus*. The reduced number of spines and tubercles on *Hylaeus* pupae probably occurred as a result of reduction (Torchio & Burwell 1987) and therefore should be considered a derived characteristic. The chemistry of cell linings produced by these two genera (salivary material only in *Hylaeus* and a combination of salivary and Dufour's gland secretions in *Colletes*) may be yet another expression of reduction by *Hylaeus*. Therefore, deductive reasoning would suggest that the singular use of sal-

ivary materials to produce cell linings is a derived characteristic. Unfortunately, the liquid release by *Colletes* pupae and the lack of liquid release by *Hylaeus* currently cannot be compared in this context because no other studies on Apocrita are available on this subject. Several additional characteristics fall into the same category.

There is general agreement that *Colletes* and *Hylaeus* are members of the same family (Colletidae) and that *Hylaeus* represents a more advanced taxon. This relationship is based on a series of interlocking tenets currently accepted by most apoid systematists: Bees arose from sphecoidlike wasps. Bees collect pollen-oil-nectar resources, and their mouthparts have evolved to accommodate efficient collection of those food sources from an enormous number of floral designs. Apoidea evolved in tandem with small open floral designs, followed by the development of short to long corolla-tubed flowers; thus, short-tongued bees are considered less advanced than are long-tongued bees. Because bees with short glossae (Colletidae) most closely resemble their sphecoid ancestors, they may represent a primitive lineage of the Apoidea. *Hylaeus*, with the absence of leg scopae, body nearly hairless, females nesting in existing holes, wing venation reduced, etc., is considered a more advanced form than *Colletes*.

Our data presented on *Colletes*, combined with results obtained on the nesting biology of *Hylaeus* (Torchio 1984), do not support particular tenets used to establish a natural classification of apoid families based primarily on comparative studies of adult forms (Michener 1944). For example, it is very difficult to explain how such a fundamental ontogenetic characteristic as embryo rotation can vary within the family Colletidae but is retained as a constant feature of the remaining Apoidea and known Apocrita. It is also difficult to accept the notion that *Colletes*, with its short tongue and bifid glossa, is (by definition) a primitive bee after observing the adaptive abilities of only one species (*C. kincaidii*) collecting pollen-nectar resources from long corolla-tubed flowers (*Phacelia*), or from narrow corolla-tubed flowers having a tripping mechanism (*Melilotus*), or from large open flowers (*Lithocarpus*) without affecting pollen-nectar collecting efficacy.

We agree that apoid mouthparts probably evolved in tandem with flowering plants, and we concur that the long tongues of some bees have evolved in response to the appearance of long corolla-tubed flowers. We do not agree, however, that apoid mouthparts evolved primarily through selective pressures initiated when pollen-nectar resources were first used as cell provisions by ancestral apoids. In fact, our results demonstrate that *Colletes* and *Hylaeus* (Torchio 1984) use their mouthparts more extensively to construct cell linings (functional cell) than when used in the collection of pollen-nectar resources. Because cell linings are not constructed by the presumed ancestors

of the Apoidea (sphecoid wasps), then surely the derived cell lining feature of the Apoidea evolved through selective pressures not heretofore considered. These selective pressures also played an important role in establishing the morphological parameters of apoid mouthparts. If this is true, we would expect apoid mouthparts to carry convergent characteristics that could be identified through detailed studies on the nesting biology of representative bee taxa supported by chemical analyses of cell linings. Once these characteristics are identified, we may eventually learn that *Colletes* and *Hylaeus* are more distantly related than currently perceived, but together they may represent an extant apoid lineage derived from less advanced apoids whose living relatives do not construct cell linings.

Acknowledgment

Our sincerest appreciation is extended to Ella Alluisi, who allowed us access to her Bonny Doon property, directed us to a number of undisturbed nesting sites, and permitted removal of nesting blocks. Bill Hevron (California State University, Fresno) contributed greatly by helping excavate and carrying large soil blocks to the vehicle. Andy Moldenke (Oregon State University, Corvallis) first directed us to the field site and made available an unpublished manuscript. Mr. Abdul-Aal Mohamed is recognized for maximizing available bloom in the greenhouse during this study. Ellen Parker (Logan, Utah) inked figures and Jack Hall (University of California, Riverside) identified the bombyliid fly. C. D. Michener (University of Kansas, Lawrence), W. P. Stephen (Oregon State University, Corvallis), J. H. Cane (Auburn University, Auburn, Ala.), and J. Tengö (Uppsala University, Sweden) reviewed the manuscript. This is a contribution from the Utah Agricultural Experiment Station, Utah State University, Logan, Utah, Journal Paper No. 3384, and USDA-ARS, Bee Biology and Systematics Laboratory, Utah State University, Logan.

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Received for publication 31 July 1987; accepted 8 March 1988.