Research article

Silk production by adult workers of the ant *Melissotarsus emeryi* (Hymenoptera, Formicidae) in South African fynbos

B.L. Fisher[1] and H.G. Robertson

Life Sciences Division, South African Museum, P.O. Box 61, 8000 Cape Town, South Africa
[1] *Present address: Department of Entomology, University of California, Davis, CA 95616, USA, e-mail: bfisher@samuseum.ac.za*

Summary. In montane fynbos in South Africa *Melissotarsus emeryi* Forel was found nesting in live wood of *Leucospermum praemorsum* (Meisn.) E. Phillips, in association with armor-scale insects (Diaspididae). A loose network of silken material was found along gallery tunnels and was combined with wood particles to seal cracks in tunnel walls and to close exit holes. Our observations reveal that the silk is produced by adult workers from glands located in cuticular depressions on the ventral portion of the anterior margin of the hypostoma. Silk was applied in nest construction with the aid of modified protarsi in the form of "silk brushes." *M. emeryi* is the only adult Formicidae known to produce silk and is the only record of silk production at any life stage in the Myrmicinae. We discuss silk production in *Melissotarsus* in relation to nest construction, defense, and diaspidid symbionts.

Key words: Melissotarsus emeryi, silk production, Diaspididae, *Leucospermum*.

Introduction

Silk is a fibrous protein containing highly repetitive sequence of amino acids that is stored as a liquid and produced into fibers when sheared at secretion (Craig, 1997). The ability to secrete fibrous proteins is a primitive feature of the hexapods, occurring first in adult hexapods for use in reproduction (Craig, 1997). Phylogenetic comparisons across all insect taxa suggest that silk production by adult insects was lost and regained multiple times but is relatively rare and restricted to a few groups of insects (Rudall and Kenchington, 1971; Craig, 1997). However, most of the derived endopterygote insect larvae (Hymenoptera, Diptera, Siphonaptera, Trichoptera and Lepidoptera) produce labial gland silks that are used for protection (Craig, 1977).

Among Hymenoptera, production of silk-like materials by adults has been reported from only three genera of Sphecidae (*Psenulus*, *Microstigmus* and *Arpactophilus*), 2 genera of Chalcidoidea (*Eupelmus*, *Signophora*), 2 genera of Vespidae (*Quartinia*, *Polistes*) and one genus of Colletidae (*Hylaeus*) (Melo, 1997). According to Craig (1997), silk glands in adult Hymenoptera are only known to occur as derivatives of colleterial glands (accessory to insect genital glands). In larval Hymenoptera, cocoons (sometimes reduced to a lining of the larval cell) are spun with silk from labial glands in all taxa except most Chalcidoidea and Cynipoidea and some Apoidea and Formicidae (Naumann, 1991). Production of silk materials in the Formicidae has only been clearly identified among larvae in the subfamilies Aneuretinae, Apomyrminae, Cerapachyinae, Ecitoninae, Formicinae, Myrmeciiinae, Nothomyrmeciiinae and Ponerinae (Baroni Urbani et al., 1992). Adult weaver ants of the Old World Formicinae (genera *Camponotus*, *Dendromyrmex*, *Oecophylla* and *Polyrhachis*) have coopted silk produced by their larvae to build extensive and often polydomous nests in vegetation above ground (Hölldobler, 1983; Hölldobler and Wilson, 1977a, b, 1990).

Prins et al. (1975) observed a silken material in the nests of *Melissotarsus emeryi* Forel and a few years later suggested that the silk is produced by the adult ants (Prins, 1978). In this paper, we describe the location of silk production, the silk application behavior and its use in nest construction in adult *Melissotarsus emeryi* Forel. We also discuss unique morphological characters of *Melissotarsus* in relation to its nesting habits, silk use and association with armored scale (Diaspididae) symbionts.

Methods

Study organism

The genus *Melissotarsus*, which currently includes 4 poorly defined species, is known from southern Saudi Arabia (Collingwood, 1985) to southern South Africa (Bolton, 1973, 1982) and Madagascar (Fisher

et. al., 1998). Though this genus is widespread in Africa and Madagascar, it is often not collected because of its unusual habit of tunneling and nesting in live wood. Older workers have worn-down mandibles from tunneling into healthy wood (pers. obs.) and were initially described as a separate soldier class separate from a worker caste with more acute mandibles (Delage-Darchen, 1972). Delage-Darchen (1972) was the first to describe the unique fashion in which these ants move about in the galleries. The ants walk with the middle pair of legs projecting upwards in contact with the gallery roof.

Though *Melissotarsus* is a potential threat to managed forests, it has been recorded only once as a pest. Along the downtown streets in East London, South Africa in 1976, approximately 50 year old *Ficus sur* Forssk. trees were infested by *M. beccarii* (G. J. Petty, unpublished report; Ben-Dov, 1978; Prins et al., 1990). The infestation extended to the highest branches of the trees to an extent that whole, large branches had to be removed to prevent them from collapsing on pedestrians and vehicles.

Study site

Live specimens of *M. emeryi* used in this study were collected from *Leucospermum praemorsum* (Meisn.) E. Phillips (Proteaceae) located in South Africa, Western Cape Province, in the Nardouw Mountains, 23 km NE of Clanwilliam, 31°58′S, 18°51′E, at an altitude of about 500 m by B.L. Fisher and C.J. Thomas on 21 June 1997 (B.L.Fisher # 1531). The vegetation type was mountain fynbos (sensu Low and Rebelo, 1996) located on nutrient-poor acidic sandy soils derived from Table Mountain group sandstones. This is the same locality and population of *M. emeryi* discussed in Prins et al. (1975). Voucher specimens of the observed species were deposited in the South African Museum, Cape Town and the British Museum of Natural History, London.

The host plant *L. praemorsum* was common along the roadsides on the plateau and most plants were occupied by *M. emeryi*. *L. praemorsum* is tree-like shrub reaching heights of 5 m and has extrafloral nectaries on the dentate leaf tips. Trees at this study site were flowering during the June visit. *L. praemorsum* forms large populations of several thousand plants but is localized in distribution to the dry, sandy, sandstone flats of this region (Rebelo, 1995). Observations were conducted in the field at 10 trees and the branches from two trees with live colonies were removed and taken back to the lab.

Observations

Photographs were taken with a JEOL JSM 5200 scanning electron microscope. In order to work out the details of the spinning process, ants were videotaped for 60 minutes through a dissecting microscope. The entire sequence of silk application was analyzed frame-by-frame using a video player connected to a Macintosh computer and monitor.

Results

Silk production

In *Melissotarsus emeryi*, the silk is produced from a number of spigots along the cuticular surface of the posterior and lateral margins of the buccal cavity (Figs. 1–3). The spigots are located within grooves on the ventral portion of the anterior margin of the hypostoma (Fig. 2). Hairs along the outer margin of the buccal cavity (Fig. 1) curve over the spigots (Figs. 2, 3) and may be used to stimulate or initiate silk production. Silk fibers extending from adjacent grooves may be fused to form larger silk strands (Fig. 3).

Silk application behavior

Still photographs, videotape and live observations through a dissecting scope were used to view the application of silk to seal openings in galleries and exit holes. We could not observe the application of silk along gallery walls because of the reaction of the ants to disturbances to the tunnels. When a small hole in a tunnel was created for observation, workers became preoccupied with closing the opening. Only workers were observed producing silk and participating in nest construction. The nest queen, and alate males and queens that were present in the nest were never seen producing silk. The sequence of behaviors by which silk and wood particles were applied in the nest by a worker is summarized as follows.

With a wood fragment in her mandibles, an adult worker approached a section of the tunnel wall where there was an opening. Before silk was applied and during silk application, the antennae were brought forward and the flat portion of each antennal club was lowered and passed over the edge of the opening at the site of silk application. The wood particle was held in the mandibles at the position to be applied. A tuft of ventrally and anteriorly curved hairs is located in a pit medially on the external margin of the mandibles (Fig. 1) which may assist in positioning the wood particles.

Silk was then drawn out from the hypostomal silk glands (Figs. 1–3) by lifting the forelegs alternately in a kneading motion to the anteroventral region of the head. The silk brush on modified protarsal segments 2–4 (Figs. 4, 5) was used to capture silk fibers from the glands and apply them to the opening and adjacent wood particles. The modified protarsus consists of a greatly swollen and flattened basitarsal segment and distally concave tarsal segments 2–4 with numerous short, stiff, conical and filiform hairs on all segments (Figs. 4, 5). In contrast, the basitarsal segment of the mid-and hind-tarsi are swollen and tubular with an apical ring of teeth and tarsal segments 2–4 are not concave and lack numerous short hairs (Fig. 6).

To draw the silk out, the silk brush was lifted up to the underside of the head and drawn mesially and distally across the hypostoma to the buccal cavity. Up to 5 silk strands from each side of the hypostoma were pulled by the silk brush. Frame-by-frame video analysis revealed that the silk brush was not always brought in contact with the silk glands. The brush was often brought in contact with the projecting silk strands just below the gland surface. This suggests that the silk was pulled out of the glands by the action of the tarsal brush. The modified tarsal silk brush functions both to pull the silk out of the gland and to apply the silk. The basitarsal segment of each foreleg also includes two rows of short stiff hairs opposite the tibial spur (Fig. 5). During silk application, silk was never seen attached to these stiff hairs on the basitarsal segment. The comb on the probasitarsal segment was never observed in use but it could serve in grooming, such as cleaning silk from the opposite silk brush.

After an initial series of silk applications, the forelegs were alternately pushed against the wood fragment until it was dislodged from the mandibles and placed on the edge of the tunnel opening to be closed. Spinning continued until the
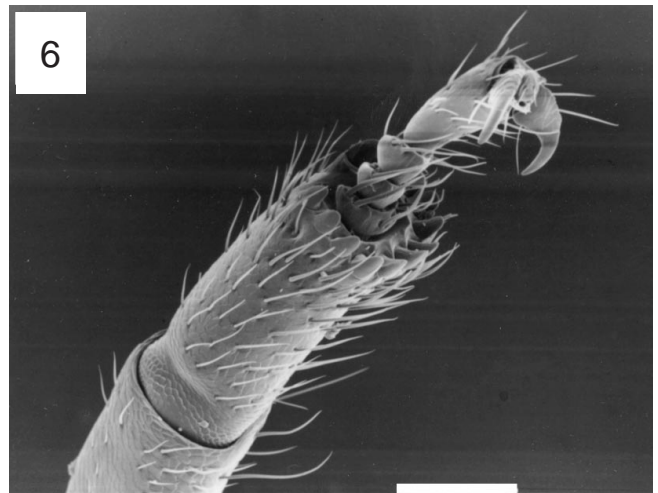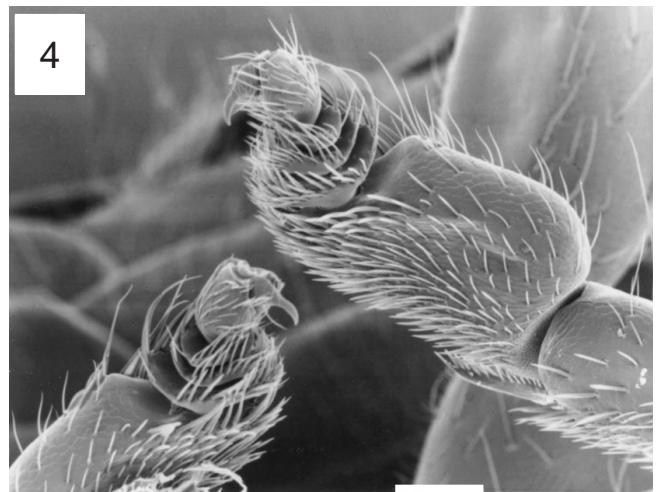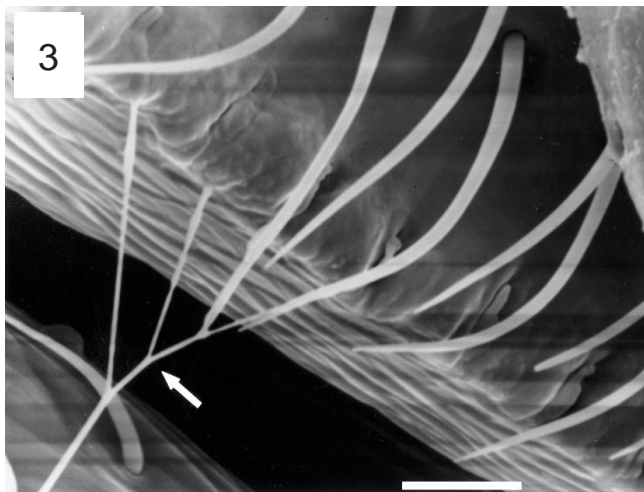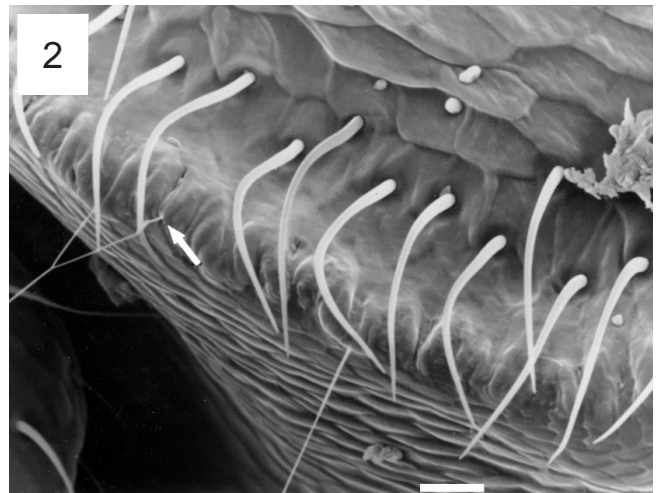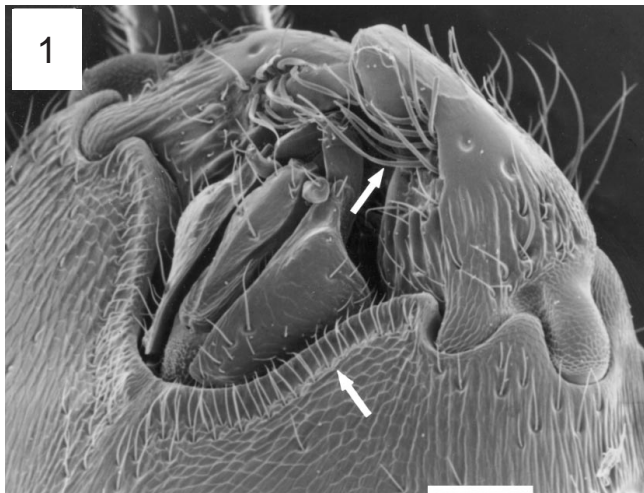
Figures 1–6. Scanning electron micrographs of *M. emeryi* showing silk producing spinnerets, protarsal silk brush and mesotarsus. 1. Ventral view of head showing buccal cavity and mandibles. Arrows indicate hairs along margin of buccal cavity and tuft of hair on mandible; scale = 100 µm. 2. Silk producing glands on the external cuticular surface of the margin of the buccal cavity, showing slits in the cuticular margin where silk fibers originate (arrow); scale = 10 µm. 3. Enlargement of the silk producing gland region, showing the fusion of silk fibers into larger strands (arrow); scale = 10 µm. 4. Modified protarsus with swollen basitarsal segment and silk brush (segments 2–4); scale = 100 µm. 5. Setal brush on protarsus, showing two rows of short stiff hairs opposite the tibial spur (arrows); scale = 100 µm. 6. Mesotarsus with swollen basitarsal segment and apical ring of teeth; scale = 100 µm

wood fragment was incorporated into the network of other fragments and silk. After the kneading motion of the forelegs had stopped, silk strands were seen still attached to the hypostomal silk glands and to the tunnel wall. These silk strands were drawn out and elongated as the ant moved from the site.

Silk use in nest construction

Nest structure consisted of 1 to 2 mm diameter tunnels under the bark with the occasional extension into the center of the trunk or branch. A loose network of silk was found along most tunnel walls. The tunnels exit to the exterior with numerous small circular openings in the bark (2 mm diameter). It has been suggested by Prins et al. (1975) that these holes are used for the release of alates but in this study workers were also observed releasing pieces of wood fragments from gallery excavations out the exit holes. When not in use, the exit holes were sealed with a network of silk and wood particles. The bark was often slit open along tunnels that ran just under the bark surface. The slits were most common at the junction of branches and trunk. Like the exit holes, these slits were sealed with wood particles held together with a loose network of silk. Overall, silk was used in three locations: along tunnels, slits and exit holes. The entire foraging area of the workers, therefore, was sealed with silk. Workers were never seen foraging outside their tunnels in the host plant, including at the extrafloral nectaries on the leaves of the host plant. Observations, however, where not conducted at night. When taken out of the galleries, the workers moved awkwardly and often could not right themselves when turned over. The second and third coxae are massively developed with the second being distinctly the largest, and appear to restrict leg movement outside galleries.

Discussion

Silk gland

The silk producing system in *Melissotarsus* appears to be derived from a different pathway than in other adult Hymenoptera. The physical, chemical and mechanical properties of the silken material produced by *Melissotarsus* are not known and we are using the term silk based on the physical resemblance to silk produced by other Hymenoptera. Silk glands in adult Hymenoptera have been known to occur only as derivatives of colleterial glands (Craig, 1977). For example, Melo (1997) provides anatomical descriptions of colleterial silk glands in female adult sphecid wasps which use silk to line nest walls and to make partitions between brood cells. Melo revealed groups of glandular cells and ducts linked to a dense setal brush associated with the 4th and 5th sterna of the females in the genus *Psenulus* while in the subtribe Spilomenina they were associated with the 6th tergum. In *Melissotarsus*, the silk glands are located along the cuticular surface of the ventral portion of the anterior margin of the hypostoma and therefore are not homologous to the colleterial silk glands (accessory to insect genital glands) in other Hymenoptera. We do not know if the silk gland in *Melissotarsus* are dermally derived or modified existing glands such as the labial gland.

Silk spinning behavior

Only insects in the Embiidina, Hymenoptera and Lepidoptera have evolved silk spinning behaviors, and do not simply secrete, deposit or eject the silk from the gland (Craig, 1997). Spinning is a process in which the protein is pulled at secretion, forcing the molecules to shear and orient into a parallel-β configuration (Craig, 1997). Larva of the most specialized weaver ants, *Oecophylla* do not spin silk. Silk protein is organized in parallel-β configuration by the adult workers which manipulate the larvae in their mandibles (see below) (Hölldobler and Wilson, 1990; Craig, 1997). In *Melissotarsus*, the vigorous kneading motion of the protarsal silk brush may serve to manipulate and spin the silk. The silk brush pulls the viscous fibers from the spigots, stretches and shears it.

After applying silk to the wood particle matrix, silk continued to extend from the silk glands as the adult *Melissotarsus* moved from the site. This suggests that the silk flow is stopped by breaking the silk strand. This is similar to the silk spinning process in Lepidoptera which stop the flow of silk by biting or breaking the silk strand (Fitzgerald, 1993).

Use of silk in nest construction in the Formicidae

Weaver ants (genera *Camponotus*, *Dendromyrmex*, *Oecophylla* and *Polyrhachis*) are one of the most abundant and ecologically dominant social insects of the Old World tropical regions (Hölldobler, 1983; Hölldobler and Wilson, 1977 a, b, 1990). The construction of communal silk nests is suggested to be a key innovation that has lead to their success (Hölldobler and Wilson, 1983, 1990). By using silk produced by their larvae, these species have been able to build extensive and often polydomous nests in vegetation above ground. Their ability to construct nests almost anywhere in the resource-rich vegetation above ground has been matched only by ants such as *Crematogaster*, *Pheidole*, *Solenopsis* and *Azteca* that build carton nest and shelters using plant fibers, detritus or soil. The most advanced grade of weaving has been achieved by the formicine genus *Oecophylla* where the larvae contribute silk from specialized silk glands (Hölldobler and Wilson, 1990).

There are three additional cases of possible silk use in ant nest construction. Baroni Urbani (1978) reported that in some Cuban species of the myrmicine genus *Leptothorax*, silk is incorporated into the "carton entrances" of the earthen nests. Baroni Urbani was not sure whether the silken material is produced by the ant larvae or obtained by the workers from some other source such as spider webs. Hölldobler and Wilson (1990) suggested that since no other myrmicine is

known to produce silk under any circumstances, it is doubtful that the silk was produced by these *Leptothorax* species. Adult production of silk was not considered. Hölldobler and Wilson (1990) also discount the observation by Jacobson and Forel (1909) that the dolichoderine ant *Technomyrmex bicolor textor* uses silk to build nests since no other dolichoderines are known to produce silk. Maschwitz et al. (1991) observed other *Technomyrmex* species in Malaysia nesting in preformed silk shelters of spiders and caterpillars and suggested that this phenomenon could have been misinterpreted by Jacobson and Forel as nests produced by the ants themselves. Most recently, Maschwitz et al. (1991) noted that isolated workers of an undetermined dolichoderine in the genus *Dolichoderus* constructed silk nests. They did not observe, however, where or how the silk was produced by the worker ants. The presence of silk production in adult *Melissotarsus* suggests that we should reevaluate silk production by *Leptothorax*, *Technomyrmex* and *Dolichoderus* workers.

Evolutionary advantages of silk

The silk in the nests of *M. emeryi* functions as a matrix of support for sealing slits in tunnels and for closing exit holes. Other ants in the same subfamily use carton material (e.g., soil, plant material) for nest construction. The selection pressures that favored silk instead of carton production by *Melissotarsus* are not obvious but may be related to the unique nesting habit or the Diaspididae nest associates of *Melissotarsus*.

Melissotarsus differs from most ants nesting in live plant tissue because they create their own nest by tunneling into live tissue. Ants that inhabit ant-plants (e.g., *Cecropia*, *Acacia* or *Triplaris*) occupy specialized and preformed nest cavities and only modify nest structure by creating entrance holes or removing soft parenchymous tissue (Bequaert, 1922; Huxley and Cutler, 1991). Ants also nest facultatively in living plants that produce a preformed cavity, such as in hollow bamboo stems.

The production of silk may allow *Melissotarsus* to make use of the living wood habitat in a way not possible by other ants species. It is possible that carton material is not effective in nest construction in living wood. The silk matrix used to seal the tunnel walls may serve to limit destruction of the host plant by the ants galleries.

Silk could also serve some defense mechanism by deterring predators and parasites of the ants or diaspidids from invading the tunnels. *Melissotarsus* is found in association with large numbers of amour scale insects (Prins et al., 1975; Delage-Darchen, 1972; Delage-Darchen et al., 1972). Prins et al. (1975) identified the armored scale living with *M. emeryi* at this study site as *Morganella conspicua* (Brain) which are present in the nest in a "scale less" form. *M. conspicua* found in the nest are without the scale that normally covers the females and the larval instars in the Diaspididae.

The protection afforded by the silk may be related to the highly reduced sting present in *Melissotarsus* (pers. obs.;

Bolton, 1982). Alternatively, Kugler (1979) noted that sting reduction often occurs in ant taxa that use plant-based foods and do not rely on the sting for food-gathering. Many of these genera also have a modified sting apparatus that incorporates sophisticated chemical defenses. We do not know if there is a modification of the sting apparatus for chemical defense or of other defense mechanisms in *Melissotarsus*. The production of silk by *Melissotarsus* to isolate the colony in living plants may be a sufficient defense mechanism.

Conclusion

Melissotarsus is an anomaly, possessing peculiar morphology, nest associates, manner of walking, and the unique capacity among adult workers of producing silk. In this paper, we describe the location of silk production and how silk is used in nest construction. Further research will need to investigate the possible functions of the silk in relation to nest construction, defense and the diaspidid associates. In addition, the genus most morphologically similar to *Melissotarsus*, *Rhopalomastix*, should be investigated for silk production. *Rhopalomastix* is distributed throughout the Oriental and Malesian zoogeographical regions and currently contains 3 poorly defined species (Bolton, 1982). According to Bolton (1982), *Rhopalomastix* has similar nesting habits as *Melissotarsus* and many of the morphological specializations of *Melissotarsus* are present in more generalized ways.

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