Alkali Bees: Response of Adults to Pathogenic Fungi in Brood Cells

Abstract. *Female alkali bees* (Nomia melanderi) *opened sealed cells containing brood infested with* Aspergillus flavus, A. tamarii, Fusarium solani, Rhizopus *sp., or* Mucor *sp. and filled them with compact soil, thus reducing fungus sporulation. Such awareness of the condition of sealed brood is hitherto unknown among solitary bees.*

Contact between adults and larvae is considered an important preadaptation for the evolution of social behavior in bees and other insects (*1*). Such contact occurs among the social honeybees and bumblebees and in allodapine bees, all of which feed their growing larvae at intervals; it also exists to some extent among social halictine bees, most of which completely provision the cells before oviposition (mass provisioning). Adult-larval contact has not been reported in the majority of Apoidea, the numerous, solitary, mass-provisioning species that seal their cells immediately after oviposition.

Sealed cells of the alkali bee [*Nomia (Acunomia) melanderi* Ckll., Nomiinae, Halictidae] containing dead or dying brood attacked by certain fungi are frequently opened by the nesting female and compactly filled with soil, reducing sporulation of the pathogenic fungi. Such attention to the condition of the brood is of interest because, in addition to its survival value, this behavior may indicate a trend toward social behavior; other species of *Nomia (Pseudapis)* are social (*2*).

Alkali bees are native to the western United States, and populations are often established and maintained by alfalfa seed growers for alfalfa pollination. These solitary bees usually nest in dense aggregations in alkaline soils with a relatively high moisture content extending to the surface (*3*). Declines in population may be attributed to fungi and other microorganisms; *Aspergillus* spp. infested 53 percent of the cells in some areas in Oregon (*4*), and five species of fungi infested 76 percent of cells in a sample from Wyoming (*5*).

Each nest, constructed and occupied by a solitary female, typically consists of a vertical burrow leading to a group of up to 24 vertical cells (*6*). Each cell is lined by the bee with a waterproof secretion, then it is provisioned with a spheroid of nectar-moistened pollen on which an egg is laid. The cell and lateral tunnel are then immediately sealed with an earthen cap and porous plug which are normally not removed during larval development (Fig. 1). After feeding and defecating, the mature larva (prepupa) usually begins diapause, to pupate and emerge the following summer.

Twenty-three nests of alkali bees, containing 82 cells, were established for behavioral and pathological studies in three glass-sided observation chambers (51 by 66 by 0.6 cm) (*7*) filled with unsterilized soil from a natural nest site. These were kept in a Nylon screen cage in a greenhouse at Logan, Utah, from 11 June to 14 August 1968. At the end of that period the observation chambers were opened, and brood was removed for histological studies and isolation of microorganisms. Nest construction, provisioning, and larval development were readily observed and appeared normal, although relatively few cells were constructed per nest (maximum, 11 cells).

In the observation chambers, female bees removed the caps from the entrances to 10 of the 29 cells containing dead or diseased brood and compactly filled the cells with soil. Cells containing healthy brood were never opened. In the field, 19.6 percent of 87 fungus-infested cells contained soil firmly packed above the fungus. All soil-filled cells contained provisions, bearing eggs or small larvae, or contained defecating larvae infected with *Aspergillus flavus, A. tamarii, Fusarium solani, Rhizopus* sp., or *Mucor* sp. However, dead or dying brood or provisions containing three species of bacteria, four species of yeasts, or 12 additional species of fungi (*8*) and some containing the five fungi listed above were not covered with soil by the bees. Fungus-infested, unpacked cells were usually completely filled with mycelium extending up to and beyond the cell cap, the hyphae passing through the cap to the adjacent burrow and cells. There was abundant sporulation of *Aspergillus* spp., *Mucor* sp., and *Fusarium* spp. toward the upper third of the cell which became completely filled with a bundle of hyphae. By contrast, when the cell was packed with earth by the bee, a barrier was evidently formed since rarely were fungi found beyond it in the cells of the current year's brood. The complete lack of sporulation (*Rhizopus* and *Mucor*), reduced sporulation (*Aspergillus* spp.), or abnormal sporulation (chlamydospores and sprout cells instead of macro- and microconidia) observed in soil-packed cells probably resulted from a relatively anaerobic environment in the usually moist, poorly drained soil inhabited by alkali bees. Poor sporulation reduced the available inoculum for the young adults which, during their emergence the next year, may encounter the fungus-infested pockets in the nesting site.

The social halictine bees, *Lasioglossum versatum* (*7*), *Evylaeus* spp. (*9*), and *Halictus rubicundus* also fill cells containing dead or diseased brood with soil; we found 6 percent of such cells of *H. rubicundus* compactly filled with soil. The packing of fungus-infested cells appears to be a defense mechanism similar to the hygienic removal of diseased brood from cells by honeybees (*10*).

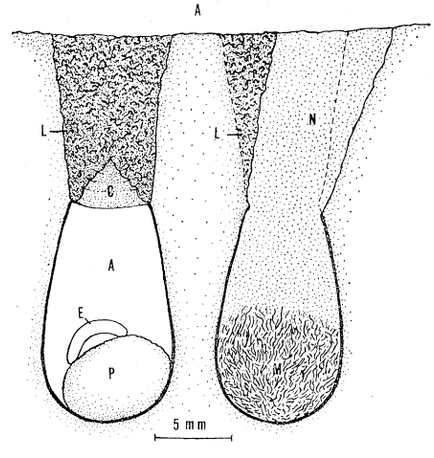

Fig. 1. Brood cells of the alkali bee. At left, normal cell with airspace (*A*) and provision (*P*) bearing egg (*E*); the cap (*C*) is in place, and the lateral (*L*) is filled loosely with soil. At right is cell in which fungus mycelium (*M*) has grown over the brood; a new lateral (*N*) was made, and it and the former airspace have been filled with compact soil.

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Turbidity, Birefringence, and Fluorescence Changes in Skeletal Muscle Coincident with the Action Potential

Abstract. *Electrical stimulation of frog striated muscle was found to produce transient changes in turbidity, birefringence, and fluorescence (of fibers stained with pyronine B). The initial phase of these optical changes was coincident with the action potential. These findings suggest that there is a macromolecular conformational change in muscle membrane during excitation.*

Contraction in skeletal muscle is known to be preceded by propagation of membrane depolarization (excitation) along the sarcolemma and the transverse tubular system (*1*). The mechanism of excitation in the muscle fiber membrane is considered to be similar to that in the nerve fiber membrane. It was recently found that the excitation process in nerve is associated with changes in fluorescence, turbidity, and birefringence (*2*, *3*). It appeared reasonable, therefore, to investigate whether there are similar optical changes in muscle fibers during excitation.

In the present studies of the optical properties of muscle fibers, semitendinosus muscles of frogs (*Rana pipiens*) were used. Under dark-field illumination, microdissection was carried out to reduce the number of muscle fibers to approximately 50. The preparation was mounted in a black acrylic chamber which allowed simultaneous electrical and optical recordings (see upper part of Fig. 1). The 2-mm long segment of muscle in the middle pool (C_1) was stimulated by a pulse of electric current (0.01 msec in duration) with the stimulus cathode in the middle pool and the anode in the lateral pool (C_2). The action potential was recorded externally with separate electrodes in the middle and lateral pools (C_1 and C_3). All these electrodes were of the silver–silver chloride type. At room temperature, 22°C, the conduction velocity of the fibers is roughly 2 m/msec; therefore, the time required to excite the entire portion of the muscle in the middle pool by a suprathreshold stimulating pulse was less than 1 msec. The pools were filled with amphibian Ringer solution.

The muscle fibers were illuminated with quasi-monochromatic light obtained with a d-c operated quartz-iodine lamp (L) and an interference filter (F_1). The incident light was focused on the muscle segment in the middle chamber from below by a × 20 objective. Changes in the intensity of light scattered by the muscle fibers were detected with a photomultiplier placed at 0° or 90° to the incident light (see D or D' in Fig. 1). In birefringence experiments, a polarizer (P) was inserted between the light source and the muscle fibers; an analyzer (A) and the photomultiplier were aligned at 0° relative to the incident light.

In fluorescence studies, a secondary filter (F_2) was placed between the muscle and the photomultiplier; light was detected at 90°. The output of the photomultiplier was connected to a Tektronix oscilloscope via a Bak electrometer stage. To increase the signal-to-noise ratio, a computer of average transients (Mnematron) was used in conjunction with a Tektronix 122 preamplifier.

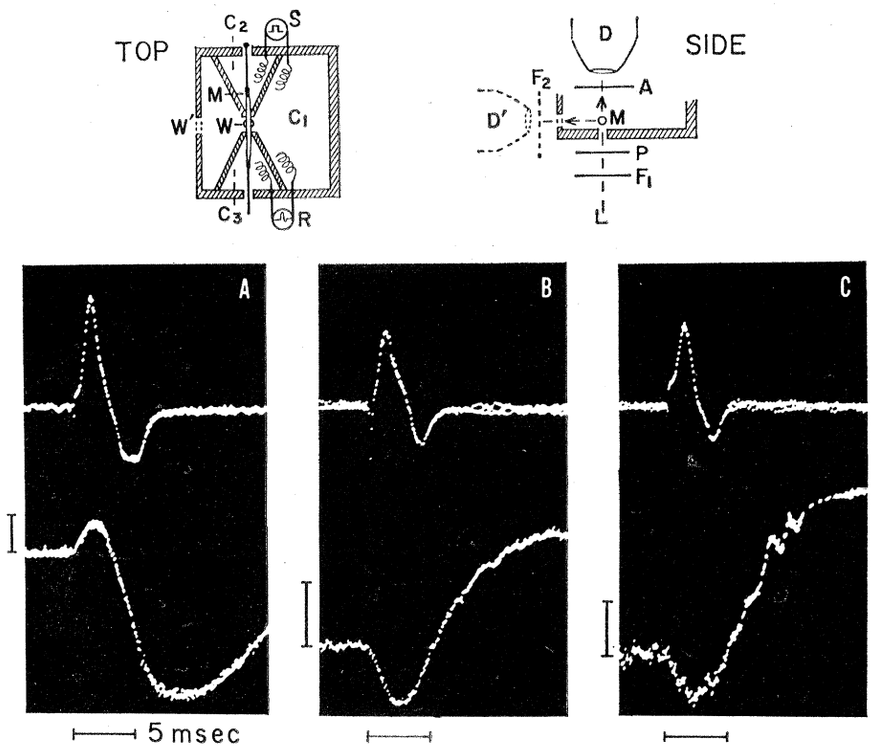

Fig. 1. (Upper) Schematic diagram (top and side views) of recording chamber and optical arrangement used for detection of changes in turbidity, birefringence, and fluorescence of skeletal muscles following electric stimulation. Abbreviations: M, muscle fibers; S, stimulating electrodes; R, recording electrodes; W and W', windows for illumination and detection; L, light source; F_1, interference filter; F_2, sharp cut filter; P and A, polarizer and analyzer, respectively; and D (or D'), photomultiplier at 0° (or 90°). (Lower) Records of the changes in light intensity (lower traces) coincident with the externally recorded action potentials (upper traces) in frog striated muscles. The records show the optical signals representing changes in light scattering (A); changes in birefringence (B); and changes in fluorescence of muscle fibers stained with pyronine B (C). Different muscle preparations were used in each record. An upward deflection of the lower trace represents an increase in light intensity. A CAT computer was used to record both the action potentials and the optical signals. The vertical bars represent an increase of 2×10^{-4} (A and B) or 10^{-4} (C) times the resting level of illumination.