DNA and RNA in the Brain of the Honeybee as a Function of Development and Age[1]

Some preliminary data showed a variation of the amount of DNA and expecially RNA extracted from brains of foraging bees. The large differences between two measurements indicated an influence not produced by technical errors. The question was whether there existed a correlation between the amount of nucleic acids and age of the bees. Our investigations cover the lifespan from the day when the larva in the cell is covered with the operculum to 51 days after the emergence of the imago. Prior to emergence all the opercula built at the same day were marked, which allowed an exact determination of the age. Young bees hatched in the incubator were marked as well and put into a beehive, where they could perform their functions that are dependent on age[2]. Animals that could not be analysed immediately, were frozen and stored in 10% glycerol on CO_2-ice. Because a small amount of RNA usually gets lost during this treatment, the data gained from the fresh and from the deep-frozen animals are shown in two separate curves from the day of emergence (Figure). After removal of the brain from the head, the compound eyes were torn off along the basement membrane. The superficial tracheae, meninges and nerve fibres were removed. Ocelli and subesophageal ganglions, as far as existent, were processed with the brain. Dissection and the following analysis were carried out on ice. For the extraction of RNA and DNA the method of SCOTT[3] was modified, as will be published elsewhere[4].

DNA. Newly covered larvae (marked −12 in the Figure) contain about half the amount of DNA of hatched animals. The pupae reach the level of the adults on day −3 (the day of emergence was taken as day 0). This finding corresponds to counts of mitosis by BÄCHI[5] who found a mean of 416 mitosis per brain on day −8, but only 13 mitosis on day −4 and 1 mitosis on day −3.

RNA. From the day of covering, the amount of RNA increases nearly linear until day −3 (Figure). The pupal exuviation takes place on day −9[5]. On day −4 the pigmentation of the compound eyes and ocelli does not differ any more from that of emerged bees, whereas the cuticula is still almost white. Values of RNA vary much more near the peak of the curve than in the ascending part (Figure). After day −2 the amount of RNA starts to decrease very fast, with a delay on the day after emergence. The mean RNA level of foraging bees in the age of 2–4 weeks is 40% lower than the one of newly emerged

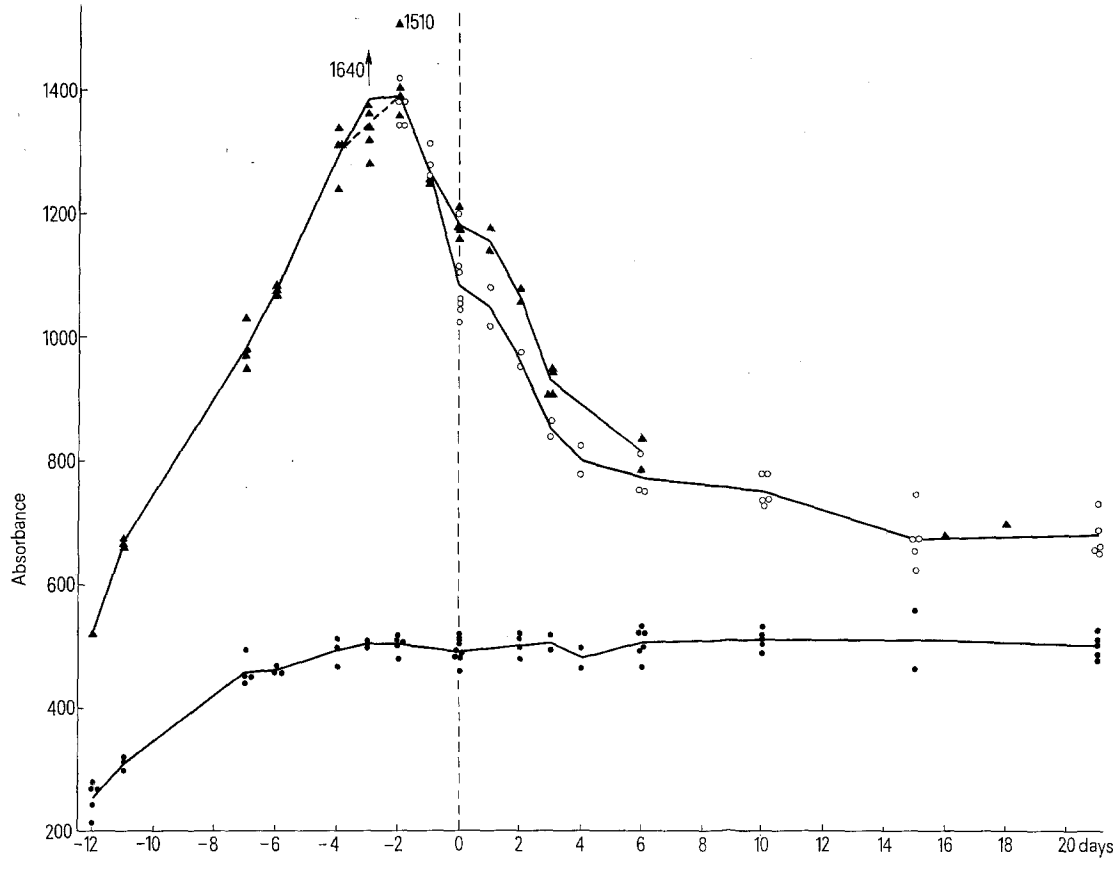
[1] Supported by Sandoz Stiftung zur Förderung der medizinisch-biologischen Wissenschaften.

[2] K. VON FRISCH, *Aus dem Leben der Bienen*, 8th edn. (Springer Verlag, Berlin 1969), p. 41.

[3] J. F. SCOTT, A. P. FRACCASTORO and E. B. TAFT, J. Histochem. Cytochem. *4*, 1 (1956).

[4] W. SALVISBERG, Dissertation Zoologisches Museum der Universität Zürich (1973).

[5] C. BÄCHI, personal communication.



Age dependence of the amount of DNA and RNA extracted from brains of honeybees. DNA: Absorbance per brain measured in 100 µl 1,6 *n* PCA. Fresh brains measured at 260 nm, deep frozen brains at 265 nm (●—●). RNA: Absorbance per brain measured in 100 µl NaOH/HCl solution at 260 nm. Fresh brains (▲—▲); deep frozen brains (○—○). The day of emergence was taken as day 0.

bees. For the period in between, KUHN[6] found a correlation between the composition of RNA and behaviour. Our results can not be compared to these findings, because the technique of gel-electrophoresis used by KUHN did not give quantitative data concerning the total RNA value of the brain.

Autoradiographical investigations have suggested, that newly emerged bees have a higher rate of synthesis than foraging bees. Therefore the decrease of RNA must be due to a higher rate of degradation of long-life or precursor RNA rather than to a decrease of the rate of recently synthesized RNA. 40–51 day-old summer bees show a second decrease in the amount of RNA of about 20%, whereas the DNA remains constant (Table).

These findings lead to three main questions: 1. Why is the biggest amount of RNA found on day −2? No more cell divisions take place at this time[5]. 2. Immediately after emergence (day 0 to 1) the decrease of RNA appears to slow down. Autoradiographical investigations might show whether the destruction of RNA is delayed, or whether the rate of synthesis of the RNA is slightly increased. 3. Why does the RNA decrease again in old summer bees? In July 1972, 2% of 350 marked bees reached an age of 50 days. The amount of RNA found in winter bees never showed a level as low as the one of 40–50-day-old summer bees (Table). Is the near end of life a reason for this second decrease of the RNA? Or is the second decrease a reason for the death of the bees? We have no control experiments on winter bees, because we do not know their exact lifespan. We suggest that the course is about the same with a much longer period of more or less constant amounts of RNA[7].

Summer bees compared to winter bees

	Summer bees Age (days) 15–20	21–30	40–51	Winter bees (Nov.-March)
RNA $\bar{x}$	680 ± 36	697 ± 41	547 ± 65	711 ± 56
n	7	7	6	44

Classes of age (days after emergence). Mean amount ($\bar{x}$) of RNA per brain, measured as absorbance at 260 nm in 200 µl NaOH/HCl solution per brain.

Zusammenfassung. Aus Gehirnen von Bienen verschiedener Altersstufen wurden DNS und RNS extrahiert und deren Mengen spektrophotometrisch bestimmt.

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[7] The authors thank Miss T. EGLOFF for technical assistance.

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Chromosomes and Giemsa-Bands of the Idaho Spotted Ground Squirrel, *Spermophilus brunneus* (Howell)

The ground squirrel subgenus *Spermophilus* in North America consists of 8 species: *S. townsendii*, *S. washingtoni*, *S. brunneus*, *S. richardsonii*, *S. armatus*, *S. undulatus* (= *parryii*), *S. columbianus*, and *S. beldingi*[1]. Earlier investigators arrived at different views concerning the subspecific or specific status of taxa now ranked within *S. townsendii* and *S. richardsonii*, and the composition of various supraspecific groups[2,3]. Relevant to this report, DAVIS[3] recognized 2 species-groups within the subgenus *Spermophilus* occurring in Idaho: 1. big-eared ground squirrels; *S. beldingi*, *S. richardsonii aureus*, *S. columbianus*, *S. armatus*, and *S. brunneus*; 2. short-eared squirrels; *S. mollis* (= *townsendii*). DAVIS[3] differentiated *S. brunneus* from *S. townsendii* on the basis of its shorter and coarser pelage, absence of a white ventrolateral stripe, certain cranial characters, the conspicuously larger ears, and spotted brown dorsal coloration. However, HOWELL[2] previously concluded that *S. brunneus*, despite its much larger ears, exhibited external and cranial characters similar to the *S. washingtoni* group, which he in turn regarded as comparing most closely with *S. idahoensis* (= *townsendii*). Recently NADLER[4] analyzed chromosomes from all species of the subgenus except *S. brunneus* and found that diploid numbers conformed to the big-eared ($2n$ = 30–36) and short-eared ($2n$ = 36–46) groups; the possibility that *S. brunneus* might belong to the short-eared group or occupy an intermediate position within the subgenus was postulated.

The present paper describes the chromosomes of *S. brunneus*, compares the giemsa-band pattern of that species with *S. townsendii mollis* which shares a similar $2n$ of 38 with *S. brunneus*, and discusses the evolutionary implications of the data.

Materials and methods. The following specimens were examined: 1. *Spermophilus brunneus* (Howell), Idaho, Adams Co., 3 miles south and 1.5 miles east of Bear Post Office, 3 females and 5 males; *Spermophilus townsendii mollis* (Kennicott), Idaho, Cassia Co., Burley, 3 females and 3 males.

Chromosomes were analyzed from femoral bone marrow and giemsa-band preparations were made from marrow cell suspensions according to SEABRIGHT[5]. Giemsa-band patterns, based on 5 mitotic figures from a male *S. brunneus*, one cell from a female *S. t. mollis*, and 5 from a male *S. t. mollis*, were diagramed to represent the composite results for each species. The bands were consistent from cell to cell within a species, although bands of certain chromosomes sometimes stained lightly or were indistinct in individual cells.

Results. All *S. brunneus* had a $2n$ = 38 and karyotype containing 14 metacentric, 16 submetacentric, and 6 sub-

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