

**EFFECT OF SOME PECULIARITIES OF
TRYPTOPHAN METABOLISM ON HONEYBEE
(*APIS MELLIFERA* L.) SENSITIVITY TO THE
QUEEN PHEROMONE**

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Key words. Mutations of locus snow and ivory, tryptophan, kynurenine, ontogenesis, nerve system, chemoreceptors, behaviour.

Summary. Ontogenetic dynamics of honeybee sensitivity to the queen pheromone at 10⁻¹ queen equivalent has been investigated at behavioural and distant chemoreceptor levels. Bees of Carpathian race, wild type, mutations of locus snow (redundant accumulation of tryptophan and serotonin with absence of kynurenine in the organism) and mutations of locus ivory (redundant accumulation of kynurenines) have been used. Redundant amount of tryptophan in bee's organism in the early stages of ontogenesis was determined to inhibit bee's sensitivity to the queen pheromone. This is equally manifested both at receptor and behavioural levels. If the content of kynurenines in bee's organism is redundant, its sensitivity to the queen pheromone at the behavioural level does not undergo

any changes during various stages of ontogenesis. At the receptor level, however, the sensitivity experiences great alterations: it is low in 1-day-old bees, reaches maximum in 7-10-day-old ones and decreases afterwards.

Triptofano metabolizmo ypatybių poveikis medunešių bičių (*Apis mellifera* L.) jautrumui motinos feromonui

Reziუმė. Bičių darbininkų jautrumo motinos feromonui (jos dozė 10^{-1} ekvivalento) ontogenetinė dinamika tirta pagal chemoreceptorių atsakus ir individo elgesį. Naudota karpatų rasės bičių fenotipas, mutacijos lokuso snow (organizme yra triptofano ir serotonino perteklius, nėra kinureninų), ir lokuso ivory (kinureninų perteklius). Nustatyta, kad jeigu bitės organizme susidaro triptofano perteklius, tai ankstyvose jos ontogenezės stadijose jautrumas motinos feromonui būna nuslopintas. Tai vienodai veikia receptorius ir elgesį. Esant organizme kinurenino pertekliui, bitės elgesys motinos feromono atžvilgiu ontogenezėje nesikeičia. Tačiau chemoreceptorių jautrumas būna didelių pokyčių: vienos paros bičių chemoreceptorių jautrumas mažas, 7-10 parų - didžiausias, o po to vėl sumažėja.

Влияние особенностей метаболизма триптофана на чувствительность медоносной пчелы (*Apis mellifera* L.) к феромону матки

Резюме. Онтогенетическая динамика чувствительности медоносной пчелы к феромону матки в дозе 10^{-1} ее эквивалента изучена на поведенческом уровне и на уровне дистантных хеморецепторов. Использованы пчелы карпатской расы дикого типа, мутации локуса snow (избыточное накопление в организме триптофана и серотонина, отсутствие кинуренинов), и мутации локуса ivory (избыточное накопление кинуренинов). Установлено, что избыточное содержание триптофана в организме пчелы на ранних стадиях онтогенеза угнетает чувствительность ее к феромону матки. Это одинаково проявляется как на рецепторном, так и на поведенческом уровнях. При наличии избыточного содержания кинуренинов в организме пчелы, чувствительность ее к феромону

матки на поведенческом уровне не меняется на разных стадиях онтогенеза. Однако на рецепторном уровне терпит большие изменения у односуточных бывает низкой, у 7-10-суточных достигает максимума, а после этого падает.

Introduction

Tryptophan is essential aromatic amino acid, a source of many neuroactive compounds: serotonin, tryptamine, kynurenines, causing an increased interest in it and its metabolites. In spite of numerous tests done, the biological role of these compounds in the organization of behaviour has not been fully revealed. In this aspect insects, a honeybee included, are very poorly investigated.

Genetical models - mutations of kynurenine pathway of exchange of tryptophan (КРЕТ), which lead to chronic alterations in the content of tryptophan and/or its metabolites are used as one of approaches to study the role of tryptophan and its metabolites in honeybee behaviour and nerve system activity (Пономаренко, Лопатина, Маршин и др., 1975). Disturbances in КРЕТ caused by mutation or pharmacological effects appeared to have influence on foraging and signal behaviour, conditioned responses, background bioelectric activity of thoracic ganglion, excitability thresholds of peripheral nerve system, etc. (Лопатина, Чеснокова, Пономаренко, 1985).

Questions of chemoreception are also touched upon. It was shown that

biochemical changes caused by mutation snow accelerate the process of modification of conditioned feeding reflexes to scents of lavender and carnation (Лопатина, Долотовская, 1984) and change receptor sensitivity to queen pheromone as well as to bee sting apparatus (Скиркявичене, Чеснокова, Скиркявичюс, Лопатина, 1988). We failed to obtain more data on regularities of changes in honeybee sensitivity to pheromones and other odours subject to functional state of individuals conditioned by genetically determined biochemical state.

The aim of the present work is to study the influence of tryptophan and its neuroactive metabolites, kynurenine in particular, on worker bee sensitivity to queen pheromone.

Materials and methods

Genetical models were used for the solution of the task. Tests were done on worker honeybees of wild type Carpathian race (+/+) which served as a genetical background of mutation КРЕТ in locus snow and ivory.

Mutations in locus snow provoke a loss in activity of enzyme tryptophanoxygenase (the first enzyme of КРЕТ) causing a great accumulation of

tryptophan and serotonin and total absence of kynurenines and ommochromes in the hemolymph of mutant individuals (Dustmann, 1968). Bees were white-eyed while those of wild type had dark brown eyes.

Mutations in locus ivory bring about a sharp rise in the activity of the enzyme kynurenine-3-hydroxylase. As a result, accumulation of redundant quantity of kynurenine together with traces of kynurenine acid - a derivative of kynurenine as well as absence of 3-hydroxy kynurenine and ommochromes is observed. Contents of tryptophan and serotonin conform to norm. Bee eyes are pink or redish. Heterozygous individuals (mutations ivory^{umber}) were used in the present study.

Ontogenetic dynamics of worker bee sensitivity to the queen pheromone has been investigated using electrophysiological and behavioural methods.

Magnitude of electroantennogram (EAG) in mV recorded from isolated antennae served as electrophysiological indicator. Ethanol extract of 2-year-old mated bee queen at dose of 10^{-1} queen equivalent was employed as a stimulus, this dose chosen as eliciting the greatest EAG amplitudes in worker bees (Скиркявичене, 1988). EAG recording routine has been described earlier (Скиркявичене, 1988).

The number of bees accumulating round the queen, i.e., present in the queen retinue, served as a behavioural indicator.

Counting was done daily each 10 min in the course of 1 h from 10 to 11 a.m. beginning with 1-day-old and completing with 20-day-old imago. This period of time has been chosen based on long-term observations which determined the hours of maximum activity in worker bees in relation to the queen under conditions of our experiment. We counted only those bees which were head-oriented towards the queen, i.e., in close vicinity to it.

Approximately 100-200 individuals were kept in a specially constructed observation hive with a constant temperature 30°C. A one-sided comb was abundantly supplied with honey, bee-bread and open brood. The observations were chiefly made on two hives parallelly. One hive contained bees of wild type, the other - mutants snow or mutants ivory^{umber}.

For each age group, 2-4 observations of queen retinue size were accomplished and 4-37 worker bees were used for EAG recordings.

The results underwent statistical processing at significance level $P \leq 0.05$. The curves were smoothed by weighted moving average.

Results and discussion

The obtained results revealed the behaviour of bees of wild type in relation to the queen in ontogenesis to change in the way determined earlier (Скиркявичюс, Скиркявичене, 1979). 3-day-old

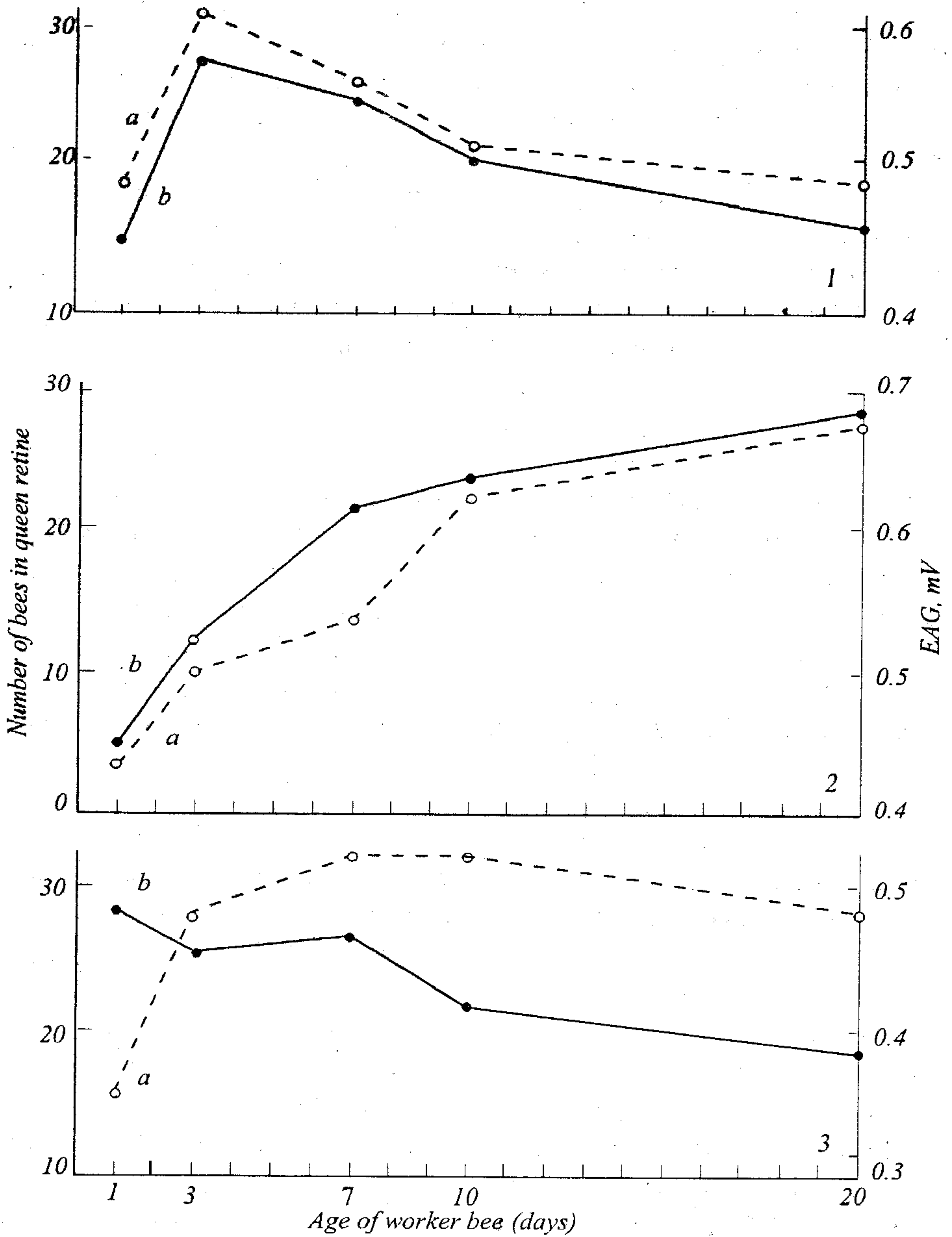


Fig. EAG of worker bees of different genotypes elicited by the queen extract:
 1 - control, 2 - mutants snow, 3 - mutants ivory^{umber}, a - EAG, mV, b - number of worker bees
 comprising the queen retinue.

bees were attracted in greatest quantities (Fig.). Henceforth, their activity towards the queen decreased remaining unchanged till the end of the observation, i.e., until the age of 20 days.

In mutants snow, the activity peak appeared to shift towards the age of 7 days. Further on, retinue size remained high surpassing the level of control bees of the same age 1.8 times in 20-day-old bees.

In mutants ivory^{umber}, a high level of queen retinue size settled down beginning with 1-day-old bees and was maintained till the age of 20 days. Mean size of queen retinue during 1 h for mutants ivory^{umber} did not differ from control bees of wild type in any period of their age (Fig.).

Comparison of electrophysiological data (Fig.) and behavioural observations of control bees in ontogenesis have revealed a striking positive correlation mentioned by other authors earlier (СКИРКЯВИЧЮС, СКИРКЯВИЧЕНЕ, 1979).

Very similar results in relation to electrophysiological and behavioural data have been obtained with mutants of locus snow.

However, this cannot be said about the obtained results using mutants of locus ivory^{umber}. In this case, the EAG amplitude increases gradually with bee age reaching its maximum and reducing slightly.

Besides, 1-, 3-, and 20-day-old mutants of ivory^{umber} exhibit a lower

receptor sensitivity to the queen pheromone than the individuals of wild type. Consequently, ontogenetic change in sensitivity to the queen pheromone is observed in mutants of locus ivory^{umber} at receptor level, without such alterations, though, at behavioural level.

Irrespective of this, ontogenetic delay in the growth of sensitivity to the queen pheromone is clearly distinguished in electrophysiological investigation of mutants.

Based on the obtained results and available literature (ЛОПАТИНА, ЧЕСНОКОВА, ПОНОМАРЕНКО, 1985) we conclude that inheritedly conditioned disturbance of tryptophan metabolism by kynurenine pathway leads to change in dynamics of formation in ontogenesis of honeybee sensitivity to the queen pheromone.

Redundant accumulation of tryptophan and serotonin and absence of kynurenines (mutation snow) in the hemolymph results in suppression of bee sensitivity to the queen pheromone in early stages of ontogenesis. In later stages, however, this sensitivity was noted to rise.

The accumulation of kynurenine (mutant ivory^{umber}) in the hemolymph had quite a complex influence on bee behaviour and operation of their antennal chemoreceptors. On the basis of our data, a conclusion can be drawn that additional studies are required for a more thorough interpretation of the obtained results.

Phenomena of delay and acceleration in maturation of functional possibilities of nerve system in ontogenesis in

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