related in structure to the farnesyl methyl ether used in several studies with positive results on cockroaches[2,7,8], showed the lowest activity of all substances examined. The activity of all aromatic compounds tested was also relatively low[9].

In summary, the cockroaches seem to maintain sensitivity to juvenoids for a longer portion of the last larval instar than many other Hemimetabola. The larval development may be prolonged considerably by repeated applications of juvenoids. *N. cinerea* responds to diverse juvenoids but their activities differ several thousand times.

*Zusammenfassung.* Mit 18 an Larven der Schabe *Nauphoeta cinerea* getesteten JHA wurden Juvenilisationseffekte erzielt. Das letzte Larvenstadium ist während 85% der Stadiendauer sensitiv. Frisch gehäutete Tiere reagieren auch auf das Lösungsmittel (Aceton) allein. Durch wiederholte Applikation von JHA können mindestens 3 zusätzliche Larvenstadien erzeugt werden, die sich zu Imagines mit abnorm langen Flügeln häuten.

WAFAA RADWAN[10] and F. SEHNAL[11]

*Entomological Institute, Czechoslovak Academy of Sciences, No Folimance 5, CS-12000 Praha 2 (Czechoslovakia), 19 October 1973.*

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[10] Permanent address: Department of Entomology, Faculty of Science, Ain-Shams University, Cairo, Egypt.
[11] We acknowledge with many thanks the critical reading of the manuscript by Dr. V. J. A. NOVÁK.

## Reduction of Biochemical Polymorphisms in Honeybees (*Apis mellifica*)

For several years electrophoresis has been extensively used for the study of isozyme polymorphisms in many different organisms. A great number of polymorphic loci has been detected in different animals throughout the animal kingdom. The study of biochemical polymorphisms in honeybees (*Apis mellifica*) presented here, was to determine if their genetic system of parthenogenesis has an impact on the percentage of polymorphic loci present in the population. The enzymes malate dehydrogenase (MDH), phosphoglucomutase (PGM) and esterase were tested. They are known to be polymorphic in other species[1-5].

For electrophoresis tissue extracts were made of entire thorax muscles (MDH, PGM) from individual workers and drones. Thorax muscles of workers were homogenized in 0.2 ml of distilled water, of drones in 0.5 ml of distilled water and then centrifuged at 30,000 *g* for 20 min. The clear supernatant material, which had a protein content of 25 mg/ml, was used for electrophoresis on cellulose acetate strips (8 × 8 cm). Haemolymph samples were taken from the dorsal vessel of individual bees and used directly for electrophoretic separation of esterase. Electrophoresis was carried out for all enzymes in a Shannon Universal apparatus at + 5 °C.

Malate dehydrogenase — 5 µl of the tissue extract were applied to the gel-strips and electrophoresis was conducted at 8 mA/240 V for 30 min using a 0.1 *M* veronal-Na buffer (pH 8.4). For enzyme detection, the strips were incubated in the dark at 37 °C in a solution of 0.2 *M* DL-malic acid, 0.001 *M* DPN, 0.000163 *M* phenazine methosulfate, 0.00043 *M* NB-tetrazolium and 0.05 *M tris* (ph 7.0). For the reaction mechanism, see BREWER[6].

Phosphoglucomutase — 5 µl of the tissue extract were applied to the strips and electrophoresis performed at 7 mA/100 V for 80 min using a 0.1 *M tris*-maleic acid buffer (ph 7.6). For staining the strips were incubated at 37 °C in the dark in a solution of 0.0046 *M* glucose-1-phosphate, 0.01 *M* $MgCl_2$, 0.0012 *M* TPN, 0.00033 *M* phenazine methosulphate, 0.00024 *M* MTT-tetrazolium, 0.075 mg G-6-PD/100 ml staining solution and 0.03 *M tris* (ph 8.0).

Esterase — 3 µl of haemolymph were applied to the strips and electrophoresis performed at 7 mA/150 V for 30 min using a 0.3 *M* boric acid buffer (pH 8.0). For enzyme detection the strips were incubated at room temperature in the following staining mixture: 0.006 *M* α-napthyl acetate, 200 mg Blue RR salt/100 ml staining solution and 0.08 *M tris* (pH 7.0)[6].

Bees from 14 different hives were studied, including 4 different races of *Apis mellifica* (see Table). All bees proved to be monomorphic for the 3 enzymes, showing the same banding pattern in workers and drones of all colonies: 1 band of enzyme activity was detected for

Reduction of biochemical polymorphisms in honeybees (*Apis mellifica*)

| Colonies | | Enzymes | | | | | |
|---|---|---|---|---|---|---|---|
| | | PGM | | MDH | | Esterase | |
| *A. mellifica carnica* | | | | | | | |
| from Frankfurt hives | 1 | 24 W | 24 D | 12 W | 12 D | 12 W | 12 D |
| | 2 | 13 W | | 11 W | | 16 W | 7 D |
| | 3 | 54 W | 52 D | 21 W | 17 D | | |
| | 4 | 12 W | 12 D | 12 W | 12 D | 27 W | 36 D |
| from Munich hives | 5 | 22 W | 24 D | 22 W | 24 D | 21 W | 9 D |
| | 6 | 61 W | 55 D | 12 W | 12 D | 12 W | 12 D |
| | 7 | | | 12 W | | 10 W | |
| | 8 | 10 W | | 6 W | | | |
| | 9 | | | 12 W | | | |
| | 10 | | | 12 W | | | |
| | 11 | 6 W | | 5 W | | | |
| *A. mellifica ligustica* | 12 | 12 W | | 12 W | | 12 W | |
| *A. mellifica caucasica* | 13 | 12 W | | 12 W | | 9 W | |
| *A. mellifica fasciata* | 14 | 12 W | | 12 W | | 10 W | |

W, workers; D, drones.

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MDH and esterase, 2 bands for PGM. This result was not to be expected considering the frequent electrophoretic variants of enzymes found in other organisms.

If compared to other species, there seem to be fewer polymorphic loci in the honeybee. What may be the explanation for this reduction? It is known that parthenogenetic reproduction should generally lead to a reduction of genetic variability. However, the enzymes of the parthenogenetically reproducing *Daphnia magna* have been shown to be as polymorphic as those of other species[7]. Honeybees, on the other hand, have a special parthenogenetic system with heterozygous workers and hemizygous drones (in *Daphnia* males are diploid). The fact that all genes are under selection in the males has to lead to a reduction in variability. Also, one of the advantages of polymorphism, namely the heterotic effect, is lost for part of the population.

Another reason for the reduction could be constancy of the environment, for bees regulate the temperature extremely well within the hive and also the feeding of the larvae. But it has been shown that animals living in presumably constant environments, like deep-sea animals and coralreef clams, have the same amount of polymorphic loci as other species[8,9]. Therefore it seems likely that the heterozygous-hemizygous system of honeybees, enforced by constant, well regulated conditions during development, and adult life is necessary to produce the observed absence in isozyme polymorphisms.

*Zusammenfassung.* Elektrophoretische Untersuchungen zum Enzympolymorphismus der Honigbiene (*Apis mellifica*) ergaben, dass alle untersuchten Individuen, Tiere aus 4 Rassen, monomorph für Malatdehydrogenase, Esterase und Phosphoglucomutase waren.

DOROTHEA BRÜCKNER

*Zoologisches Institut der Universität, Seidlstrasse 25, D-8 München 2 (Federal Republic of Germany), 23 October 1973.*

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## Chromosomes and DNA of the Ambystomatoid Salamanders

The Salamanders (Amphibia, Caudata) of the families Ambystomatidae and Plethodontidae are often associated in the suborder Ambystomatoidea[1,2]; the small paedogenetic family Amphiumidae (formerly included in the Salamandroidea[3]) appears to show various affinities with one of the preceding families[4,5]. Indeed, the members studied of the 3 families – unique within the order – have the same diploid number of 28 chromosomes with the exception of the ambystomatid *Rhyacotriton* and some bolitoglossine Plethodontids (with $2n = 26$)[6–9].

We present here new comparative data on the chromosomes and the nuclear DNA amounts in 7 species of

| Taxonomic position | Diploid number | Nuclear DNA amount (pg/N) | Reference in the Figure |
|---|---|---|---|
| Family Ambystomatidae | | | |
| Subfamily: Ambystomatinae | | | |
| Subgenus: *Linguaelapsus* | | | |
| *Ambystoma texanum* | 28 | 48 | A |
| *Ambystoma annulatum* | 28 | 50 | B |
| Subgenus: *Ambystoma*: | | | |
| The *maculatum* group: *Ambystoma maculatum* | 28 | 52 | C |
| *Ambystoma macrodactylum* | 28 | 52 | D |
| The *opacum* group: *Ambystoma opacum* | 28 | 48 | E |
| *Ambystoma talpoideum* | 28 | 62 | F |
| The *tigrinum* group: *Ambystoma tigrinum* | 28 | 55 | G |
| Family: Amphiumidae *Amphiuma means* | 28 | 150 | H |
| Family: Plethodontidae | | | |
| Subfamily: Desmognathinae *Desmognathus fuscus* | 28 | 30 | I |
| Subfamily: Plethodontinae | | | |
| Tribe: Hemidactyliini: *Gyrinophilus danielsi* | 28 | 44 | J |
| *Pseudotriton ruber* | 28 | 49 | K |
| *Eurycea lucifuga* | 28 | 41 | L |
| Tribe: Plethodontini: *Plethodon cinereus* | 28 | 46 | M |
| *Ensatina eschscholtzii* | 28 | 84 | N |
| *Aneides aeneus* | 28 | 86 | O |
| Tribe: Bolitoglossini: *Batrachoseps attenuatus* | 26 | 84 | P |