

Fig. 1. A C-banded karyotype of a female metaphase of armadillo. Note the presence of centromeric heterochromatin in every pair of chromosomes. Pairs Nos 4 and 7 have terminally located C-bands in the long arms whereas pair No. 6 has terminally located C-bands in the short arms. Pair No. 9 has polymorphic C-banded segments.

Fig. 2. A G-banded karyotype prepared from a female metaphase plate. Each pair of chromosomes has its own distinctive banding pattern. The identification of the 2 X chromosomes is not certain.

single species of the pangolin, *Manis pentadactyla*, has a diploid number of 36^{7,8}. The diploid number of 20 for the armadillo is, therefore, the lowest among these animals. Undoubtedly numerous rearrangements must have taken place in the karyotypic evolution among these animals.

Using the microspectrophotometric measurement technique to estimate the DNA content per cell, Benirschke et al.², showed that the armadillo nuclei had 1.673 more DNA than human nuclei. In eutherian mammals, the DNA content per cell is more or less the same. In cases where a species may have a higher DNA content than its related species (e.g., *Peromyscus eremicus* vs *P. crinitus*), the difference can always be attributed to the amount of C-band⁹. Since the armadillo chromosomes do not display an unusual amount of constitutive heterochromatin, the unusually high DNA content in Benirschke's material requires confirmation.

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Hemolymph volume determination in the tomato fruitworm, *Heliothis zea*

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Summary. The hemolymph volume of *Heliothis zea* larvae was determined by the amaranth dye method and found to vary from 28 to 288 μ l. It averaged 30.6% of b.wt, a value comparable to that obtained with C¹⁴ inulin. Amaranth became evenly distributed in the hemocoel in 3–5 min.

In the course of physiological and toxicological studies on insects, it is often useful to know the volume of the hemolymph. Reported here is a modification of the dye dilution method^{1,2} applied to immature tomato fruitworms (TFW), *Heliothis zea* (Boddie). Workers in this area consider this to be a reliable technique^{3,4}.

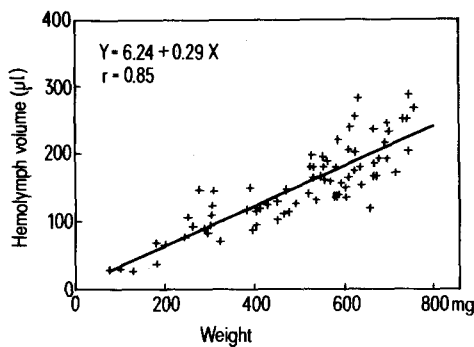
Methods. TFW larvae were raised singly in 60-mm plastic petri dishes on an artificial diet (Bio-Serv®, Frenchtown, N.J. 08825). Larvae were selected from mid-stadia of the last 3 instars for experimentation. Individuals were injected with 10 μ l of an amaranth dye solution containing 20 mg dye/ml aqueous NaCl. Injections were made under a 60 \times binocular microscope using a microhypodermic needle made from a 50- μ l disposable glass pipet drawn to a tip diameter of ca. 250 μ m. The needle was connected to a 100- μ l calibrated glass syringe via segments of polyethylene tubing of decreasing diameters. Larvae were restrained during injection by being partially wrapped in tissue paper and held between the thumb and forefinger. The injection

site was the dorsal vessel between the 11th and 12th body segments. This location was chosen because it avoided loss of dye and hemolymph (in case of loss, the larvae was discarded) and it facilitated the rapid dispersal of the dye. During dye circulation, the animal was weighed. The dye circulation period could be decreased by gently massaging the specimen. Within 5 min of injection, a 10- μ l aliquot of dyed hemolymph was collected from a severed proleg using a disposable micropipet. It was then mixed in 1.0 ml of a 0.5% sodium dodecyl sulfate (SDS) solution in distilled water, which lysed the cells and cleared the sample. Centrifugation was not found to be necessary for consistent results. Standards containing from 0.5 to 40.0 μ g dye and 10 μ l of untreated hemolymph per ml of SDS solution behaved in accordance with Beer's Law, when read at 515 nm. Samples and standards were read on an American Optical Spec 20® spectrophotometer.

Results. Amaranth dye became evenly distributed in about 3 min after injection. Hemolymph volume was calculated

using the equation of Lee¹: $V = (dg_1/g_2) - a$, where V is the hemolymph volume, g_1 is the weight of the dye injected, g_2 is the weight of the dye recovered, d is the volume of the sample, and a is the volume of the injection.

Data from 76 caterpillars were pooled and subjected to a linear regression analysis (figure). The means of the weights and hemolymph volumes from these observations were used to calculate the volume % by using the equation:



Linear regression analysis of *H. zea* hemolymph volume vs weight.

$100 V/W = \bar{V}\%$. This figure was 30.6%. The hemolymph volume of a larva of known weight can be calculated from the equation for the regression line (figure), or obtained from a $\bar{V}\%$ estimate.

Discussion. The values obtained with dye are in close agreement with those of Burton et al.⁵ who applied the C^{14} inulin method of Wharton et al.⁶ to TFW last instar larvae. The dye-technique has the advantages of being accurate, inexpensive, reproducible, and it requires little in the way of sophisticated equipment or expertise. In Orthoptera, Lee¹ determined that no dye was taken up by tissues other than the Malpighian tubules, and this began 10 min after injection. Hemolymph samples should therefore be taken between 3 and 5 min after injection to prevent loss due to staining.

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Experimental evidence of sexual selection based on male body size in *Jaera* (Isopoda; Asellota)

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Summary. In experiments where 2 males were in competition for mating, large males were at an advantage in *Jaera italica*, while no such selection based on male size existed in *Jaera istri*. Sexual selection is likely to be responsible for the sexual dimorphism in body size found in the 1st species, in which males are larger than females, while the latter species is sexually monomorphic.

Although Darwin regarded sexual competition as the ultimate cause of sexual dimorphism in animals, the debate is still open on the respective parts played by sexual selection and ecological adaptation in the evolution of secondary sexual characters²⁻⁴. The problem of the evolution of sexual dimorphism in body size is a good illustration of this, since it may result either from the mating system⁵⁻⁷, or from the bioenergetical constraints required by reproduction⁸⁻¹⁰. In contrast to many sexual characters, sexual dimorphism in body size can be measured, and permits comparison between species. We present here experimental results on sexual selection obtained in 2 related species showing different sexual dimorphisms in body size.

Jaera italica and *J. istri* are 2 isopodan species with the same female reproductive biology⁷. The former is sexually dimorphic for body size (males are larger than females), while the latter is monomorphic⁸. It has been suggested⁸ that sexual competition in males was responsible for a relative increase in male size in the 1st species. In order to check this hypothesis, experiments based upon a 'female choice test' were done in both species.

Jaera italica individuals originated from Risan (Yugoslavia), and *J. istri* ones were from Kladovo (Yugoslavia). Groups composed of 2 males and 1 female were constituted in each species, and placed within culture dishes with rearing medium⁹. In both species, females are sexually receptive at moulting, and pairing occurs about 3 days prior to copulation. As soon as a pair was found in a culture dish,

it was removed and the body length of the 'mated male', of the 'non-mated-male', and of the female were measured (from the front of the head to the end of the pleotelson). Groups in which a male was moulting during the period of receptivity of the female were discarded.

Results are given in the table and the figure. In *Jaera italica*, the mated male was the larger in most instances (table; figure, a). The smallest male was paired with the female in only 3 cases out of 31 and, in 2 of these cases, the body length of its competitor was very similar.

The result of the competition did not depend on the average size of males: the largest was at an advantage

Effect of the body length on the mating success of males

| | <i>J. italica</i> | <i>J. istri</i> |
|--|-------------------|-----------------|
| Number of assays where the mated male was the largest | 28* | 32 |
| Total | 31 | 50 |
| Body length in mm (mean \pm SD) | | |
| Females | 2.52 \pm 0.26 | 2.14 \pm 0.21 |
| Males | 3.10 \pm 0.39 | 2.12 \pm 0.26 |
| Mated males | 3.29 \pm 0.34 | 2.15 \pm 0.26 |
| Probability of the mean being different in males and females | 1.00 | 0.33 |

* Highly significant deviation from panmixia.