

mg iodine dissolved in 100 ml distilled water). For documentation the gel was photographed with an AGFA Ortho, 25 ASA, film.

In order to determine whether the  $\alpha$ -amylase allele on the X<sup>2</sup>-chromosome is dosage compensated in males or not, the  $\alpha$ -amylase activities in the homogenates of female and male flies were estimated in relation to the total protein content. To avoid dietary effects on the  $\alpha$ -amylase activity all flies of one series were taken from the same bottle<sup>9</sup>. In four independent series using 2 males or females respectively, flies were homogenized in 25  $\mu$ l phosphate buffer (20 mM Na<sub>2</sub>HPO<sub>4</sub>  $\times$  2 H<sub>2</sub>O, 20 mM KH<sub>2</sub>PO<sub>4</sub>, 25 mM NaCl, pH 7.4). The homogenate was adjusted with the same buffer to a final volume of 50  $\mu$ l and centrifuged for 30 min, 4°C. From the supernatant two aliquots of 12.5  $\mu$ l each were used to determine the  $\alpha$ -amylase activity by the modified 3,5-dinitrosalicylic acid reduction assay<sup>9</sup> and simultaneously the protein concentration measured according to Bradford<sup>12</sup>. For the measurement of the  $\alpha$ -amylase activity the samples were incubated for 30 min at 25°C.

**Results.** Eight different strains of *D. miranda* were screened for electrophoretic  $\alpha$ -amylase variants. Only one, strain 32/33, showed a different, faster moving allozyme (designated F), while all the other seven strains showed the same slow allozyme (designated S). The mobility differences between strain 32/33 and MPI are illustrated in figure 2. Both strains show a single band. Crosses were made in both directions between the F<sup>+</sup> and the various S-strains. The electrophoretic pattern of the  $\alpha$ -amylase variants appearing in one of these crosses is exemplified in figure 3. The heterozygote F<sub>1</sub> females show the expected double band pattern, while the F<sub>1</sub> males prove to be hemizygous, showing only one band.

Since only one  $\alpha$ -amylase gene is expressed in the *D. miranda* male genome, it appeared to be of interest whether this gene is dosage compensated. We determined the  $\alpha$ -amylase activity in crude homogenates of male and female flies<sup>9</sup> in relation to a defined protein amount (table). The obtained ratio male/female = 0.98 is close to 1, as is to be expected of the  $\alpha$ -amylase genes in males and females produce the same amount of enzyme. As a consequence the only active  $\alpha$ -amylase gene on the X<sup>2</sup>-chromosome must be dosage compensated in males.

**Discussion.** The electrophoretic analysis of the  $\alpha$ -amylase variants demonstrates that only the gene on the X<sup>2</sup>-chromosome

is expressed in males whereas the gene on the neo-Y is silent. At present we do not know the molecular basis of the inactivation of the gene on the neo-Y but we assume that this is due to the general degeneration of the neo-Y-chromosome<sup>4</sup>. Because in the male karyotype only the gene of the X<sup>2</sup>-chromosome is expressed it seemed interesting to look at the dosage compensation. It is known from other investigations that about 70% of the X<sup>2</sup>-chromosome are dosage compensated, 30% are not<sup>5</sup>. To compensate the differences in body size between males and females the  $\alpha$ -amylase activity was determined in relation to the protein content in the crude homogenates. The obtained male/female ratio of 0.98 strongly suggests that the  $\alpha$ -amylase allele in the male is dosage compensated and must be located in the 70% area of the X<sup>2</sup>-chromosome. In other *Drosophila* species the  $\alpha$ -amylase gene is autosomal. In order to learn more about the regulation of this gene experiments are under way to isolate the  $\alpha$ -amylase gene from a genomic *D. miranda* phage library.

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## Chromosomes of three *Brachymeria* species (Hymenoptera: Chalcididae)<sup>1</sup>

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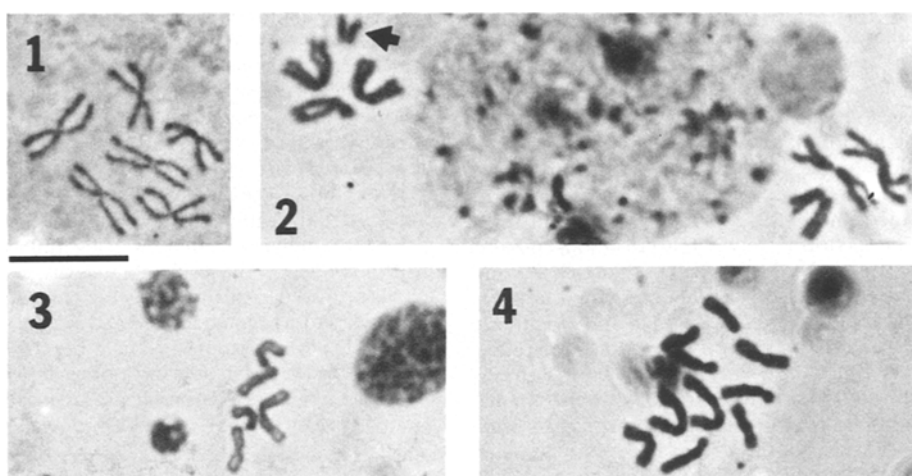
**Summary.** *Brachymeria intermedia*, a pupal parasite of the gypsy moth *Lymantria dispar*, has a karyotype of K = 2SM+1M which is the lowest number (n = 3) in Hymenoptera. *Brachymeria lasus* has K = 3M+1SM+1A (n = 5), and *B. ovata* has K = 1M+2SM+2A (n = 5).

**Key words.** *Brachymeria*; Hymenoptera; Chalcididae; karyotype.

Haploid chromosome numbers in the order Hymenoptera have been reported ranging from 3 to 42<sup>2</sup>. The lowest number of 3 is found in the ant *Ponera scabra*, although 2n = 7 (female) and n = 4 (male) were reported for this species in the original paper<sup>3</sup>. As this species involves a complicated translocation polymorphisms and no males having n = 3 were observed (H. T. Imai, personal communication, 3/23/85), the case of *P. scabra* should not be considered the lowest number in Hymenoptera in the strict sense. Here, I report the karyotype of 2n = 6 and n = 3 in *Brachymeria intermedia*, a chalcid pupal parasite of the gypsy

moth (*Lymantria dispar*), and 2n = 10 or n = 5 in two other species of *Brachymeria*.

**Material and methods.** Cerebral ganglia of early instar larvae, testes and ovaries of white pupae from laboratory cultures of *B. intermedia* (6 males, 5 females), *B. lasus* (6 males, 1 female), and *B. ovata* (6 males, 8 females, and 2 larvae) were used. *Brachymeria intermedia* was introduced from Europe as early as 1905 and has been established in North America since 1942<sup>4</sup>. The *B. lasus* culture originated from Japan. *Brachymeria ovata* is the only native species and the culture originally came from Ari-



Chromosomes of *Brachymeria* spp. Figure 1. Diploid karyotype of *B. intermedia* from brain cell of early instar larva. Figure 2. Haploid karyotypes of *B. intermedia* showing acentric segment (arrowed) resulting from breakage at the site of secondary constriction and a normal haploid

chromosome set (lower right) from spermatocyte. Figure 3. Haploid karyotype of *B. lasus* from spermatocyte. Figure 4. Diploid karyotype of *B. ovata* from brain cell of early instar larva. Scale = 10  $\mu$ m.

zona. Aceto-orcein squash<sup>5</sup> and air-drying<sup>6</sup> techniques were used in chromosome preparation. Karyotype determinations were based on at least 20 cells with well-spread chromosomes, except in *B. lasus* where only 6 cells from one male were used.

**Results and discussion.** Four submetacentric (SM) and two metacentric (M) chromosomes were observed in both somatic and germ cells of females in *B. intermedia* (fig. 1). Although a secondary constriction was not clearly demonstrated in photographic preparations, an actual breakage was observed in two cells from testes of two males (fig. 2). However, this breakage was not found in the other four males used in this study. Although the two other species of *Brachymeria* have the same haploid number of five ( $n = 5$ ), their karyotypes are different. *Brachymeria lasus* has  $K = 3M + 1SM + 1A$  (fig. 3), but *B. ovata* has  $K = 3SM + 2A$  (fig. 4).

The lowest chromosome number in animals so far found is in the false spider mites<sup>7</sup>, with  $n = 2$ . Recently, Ferreira et al.<sup>8</sup> also found  $n = 2$  in the *Chalcidolepidius* beetle which represents the lowest chromosome number in Coleoptera. The haploid number of five reported here for both *B. lasus* and *B. ovata* is the modal value for the superfamily Chalcidoidea<sup>9</sup>. Goodpasture reported  $n = 4$  in *Monodontomerus obscurus*<sup>10</sup>. Although a haploid number of three has been found in the bee *Hesperandrena duboisi*<sup>11</sup>, this report of  $n = 3$  in *B. intermedia* is the first published docu-

ment of the lowest chromosome number in the order Hymenoptera.

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## New contribution to the study of chromosomes of the European Cryptocephalinae (Coleoptera, Chrysomelidae)

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**Summary.** The chromosomes of three species of *Pachybrachis* and nine of *Cryptocephalus* chrysomelids were analyzed. The male meiotic bivalent formula of *P. azureus* Suffr., *P. catalanicus* Burl. and *P. petitpierrei* Daccordi is  $7^{II} + Xy_r$ . *Cryptocephalus sexmaculatus* Ol. and *C. vittula* Suffr. have  $13^{II} + Xy_p$ , *C. bipunctatus* L.  $14^{II} + Xy_r$ , *C. ochroleucus* Steph. and *C. ocellatus* Drap.  $14^{II} + Xy_p$ , *C. crassus* Ol.  $15^{II} + Xy_r$ , *C. sulphureus* Ol.  $15^{II} + Xy_p$ , the same number as in *C. fulvus* Goeze with  $2n = 32$  chromosomes, while *C. primarius* Har. has  $19^{II} + Xy_p$ . The modal chromosome number in *Cryptocephalus* is  $2n = 30$  (about 60% of spp.), and most species are characterized by their small chromosomes. The low variation found in the karyotypes of Cryptocephalinae along with their possible interrelationships with allied chrysomelid subfamilies are also discussed.

**Key words.** Chromosomes; cytogenetic evolution, Cryptocephalinae; chrysomelid beetles.