Fertile diploid drones in africanized honeybees, Apis mellifera adansonii

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Summary. 59 diploid drones of Apis mellifera adansonii, 12–37 days old, were tested for the presence of semen after provoked ejaculation; 13 drones ejaculated semen enough to be used in an instrumental insemination, but only three of them (5%) furnished 1 mm^3 of semen. The problems referring to the attainment of descendants from the 2n drones are briefly discussed.

Homozygosity of X alleles in Apis mellifera causes reduction in the size of the testes of diploid drones and the inheritance of this character is in accordance with the additive action of polygenes[1-3]. This paper reports data about the percentage of fertile diploid drones in Africanized honeybees (Apis mellifera adansonii). 59 diploid drones of Apis mellifera adansonii were reared by Woyke's method[4]. These drones (12–37 days old) were tested for the presence of semen on the penis tip, after provoked ejaculations. The males were marked on the first day of life (with special paint on the thorax) and maintained in a strong queenless colony until the experiment. This colony was housed in a Langstroth nest with 9 frames, 6 with sealed brood and 3 with honey and polen.

Haploid drones of Apis mellifera, 8–10 days old, are sexually mature[5,6] and ejaculate an average of 1.7 mm^3 of semen, this volume being equivalent to 11 million sperms[7]. Camargo[8] found 6 million sperms per mm^3 in haploid drones of Apis mellifera adansonii, apparently the only race that can produce diploid drones with large testes[2]. Honeybee queens that received from 1 to 20 mm^3 of semen in instrumental inseminations presented 1.4–5.8 million sperms in the spermateca[7]. This represents a percentage of success varying from 20 to 25% approximately. Only 13 diploid drones from our sample (22%) presented semen. These drones had the following ages: 12 days (2 drones), 13 (1), 15 (1), 17 (2), 23 (2), 25 (1), 30 (2), 33 (1), 37 (1). The volume of semen collected from the fertile 2n drones varied from 0.3 mm^3 to 1 mm^3. Nevertheless only three of the thirteen fertile drones ejaculated 1 mm^3 of semen (approximately 5% of the sample). Therefore, practically only these 3 drones would have a chance to produce descendants. These facts explain why it is so difficult to obtain descendants from diploid drones of Apis mellifera[9].

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Light-dependent homosexual activity in males of a mutant of Drosophila melanogaster

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Summary. Cytogenetical and behavioural studies on a γ-ray-induced mutant of *Drosophila melanogaster* is reported. The males of this mutant show abnormal phototactic response and light-dependent homosexual activity.

Sexual activity in males of *Drosophila*, like in most other animals including man, is a complex innate behavioural process which can be partitioned into a) act of pre-copulatory courtship and b) copulation. A sexually active *Drosophila* male performs acts of courtship consisting of a) recognition of female and orientation, b) wing vibration which is species specific and c) licking of female genitalia and attempted copulation, in a predictable order[2-5]. The first element of this series, the recognition of the female, is brought about through the agency of sex-pheromones emitted by mature females[6]. Thus, the sexual arousal in males is olfactory in nature and visual or other factors[2] do not seem to play a major role in the sexual activity of males of *Drosophila melanogaster*. In this communication, an interesting case of light-dependent sexually abnormal behaviour of males of a mutant of *Drosophila melanogaster* is reported. This mutant arose during experiments designed to isolate abnormal phototactic mutants. In these experiments, one-day-old Oregon-K males were irradiated with 3 kR of γ-rays and mated to attached-X virgin females. Resulting progeny was subjected to phototactic screening, using Benzer's[7] counter current distribution method. Flies not responding to light were isolated and designated as 'sluggish'. Males of one such sluggish mutant, besides showing abnormal

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phototactic response (Figure 1) on exposure to light, had abnormal sexual behaviour. The light-triggered sexual activity is conditional, i.e. it lasts only as long as the flies are exposed to light and is abnormal in the sense that such sexually active males do not discriminate between the sexes while performing the act of courtship. Homosexual tendency in males of a mutant of *Drosophila* has been reported by GILL[8]. More recently BENZER[9] has reported a mutant 'fruity' where males pursue each other rather than females. The mutant reported here also shows a homosexual tendency. Its dependence on light for abnormal sexual activity attracted our attention and the mutant was put to genetic and behavioural analysis.

A detailed genetic and cytological examination revealed that a) the males of this mutant are partially fertile, b) homozygous females are sluggish but sterile, c) there is complete suppression of crossing over in X-chromosome in heterozygous females (genotype +/y cv v f car), and d) the sluggish flies have a reciprocal translocation between X- and III-chromosomes. The analysis of salivary gland chromosomes showed that the points of exchange involve the X-2^{e} segment of the X-chromosome and the 3L-97$^{A\text{-}10}$ segment of the third chromosome. Thus, the observed behavioural phenotype may be due either to a gene mutation in the translocated segments, or to the position effect brought about by the transposition of the third chromosome segment to the X-chromosome. The data on genetic analysis (table), however, show that the mutant phenotype is recessive and follows a segregation pattern typical of a sex-linked character.

In order to study the influence of light on sexual behaviour, mutant as well as wild type (Oregon-K) males were maintained with attached-X females in dark. 4–5 days prior to test, virgin males and females were separated from the stock bottles and stored on normal food in dark. Behavioural studies were carried out in red and white light. The light source used was a 40 W fluorescent tube fitted at one meter distance from the observation desk. For red light a Kodak safety lamp fitted with a red filter was used. Each observation cell, a glass tube of 10 × 2.5 cm, contained about 25 pairs of virgin females and males. The observations made on sexual behaviour show that unlike normal males whose sexual activity was independent of light conditions, the sexual awakening in mutant males was light-dependent. These males did not mate in dark with mature virgin females. Exposure to

Analysis of F_2 progeny of selfed F_1 of a cross involving virgin Or-K females and sluggish males

Phototactic response of the flies	Males[a]	Females	Total
Positive phototactic	330	549	924
'Sluggish'	336[b]	24[c]	360
Total flies	666	618	1284

[a] Half of the males in F_2, segregating for mutant phenotype, suggest a sex-linked inheritance and 336:1284 ratio fits into an expected monogenic segregation. [b] Actual number of males in this class were 168 which were corrected to 336 on the presumption that 50% of the males carrying deficiencies or duplications of X or 3rd chromosomes will be lethal because of translocation. [c] These females need not be sluggish since even in a normal population, a few individuals could be expected to have a very poor phototactic response and be mistaken as sluggish.

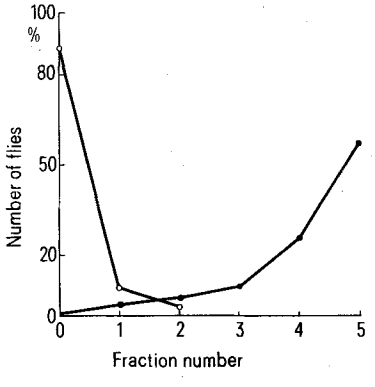

Fig. 1. Phototactic response of mutant males (○—○) and attached X-females (●—●) measured by BENZER's[7] counter current distribution method. Fraction numbers indicate the tube number where tube No. 0 is the starting tube and will retain the flies having poor light response while tube No. 5 will have the best responders.

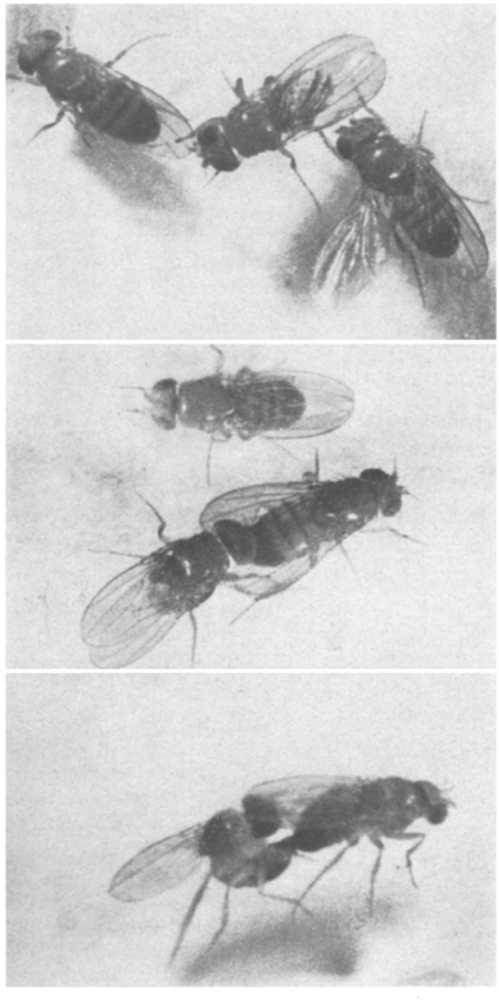

Fig. 2. Courtship behaviour in mutant males of *Drosophila melanogaster*. *a* A chain of three sexually active males, the lower one is seen vibrating a wing. *b* A male is seen beginning to mount another male. *c* 2 males seen in the process of transient pseudocopulation.

normal light, however, resulted in about 40–50%, sometimes even more, copulating pairs within a period of 10 min. Also, sexually aroused males did not discriminate between the sexes and, besides courting females, showed a persistent courting of males (Figure 2). When the observation tube had only males, or when the proportion of males to females was very high, chains or rings of 4 to 5 males courting each other was observed. Interestingly, the males being followed did not flicker their wings, a behavioural property of normal males. These observations suggest that the sexual arousal in the mutant males, unlike the normal males, is not mediated through female sex-pheromones. Instead, light probably triggers the very initial step of this complex process.

Besides the effect of normal light, observations were also made on the influence of different wave-lengths of light on the sexual activity of mutant males. For this, a rectangular (30 × 30 cm), light proof card-board box was used. It was fitted with a 100 W bulb on the top and an opening in one side wall with sliding shutter to accommodate a monochromatic filter. Monochromatic light was obtained by employing monochromatic filters – approximate band pass 30 nm and wave length coverage of 660–420 nm. Mutant males, approximately 50 in number and isolated within 6–8 h of eclosion, were kept in dark on normal food for 4–5 days. Just before observations, males were transferred, in dark, into empty glass tubes (10 × 2.5 cm) and kept at a distance of about 30 cm from the opening in the box. Flies were observed under various wave lengths of light. Each shift in wave length was preceeded by a 2-minute dark period. Sexual activity measured in terms of acts of courtship-wing vibration and attempted copulation was recorded for 5 min at each wave length. It was noticed that the red and orange part of the light spectrum (wave length 660–595 nm) had no influence on the sexual activity, though males move about normally. Yellow light (wave length 575 nm) resulted in a total of 80–90 attempts of courtship and pseudo-copulation among males (attempts made by individual males were not recorded). Sexual activity at wave lengths up to 515 nm was more or less of the same order as that in yellow light. However, wave lengths nearing violet (420 nm) could induce only 15–20 courtship and copulation attempts. Further, the abnormal sexual activity induced in yellow light (and other parts of the spectrum) could be instantaneously terminated and regained by intermittent exposure to red and yellow light. This finding is of importance and will be of great help in delineating events from the perception of the stimulatory signals through transmission to the nervous system and up to the effects on effector organs.

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New Robertsonian metacentrics in another 22-chromosome mouse population in Central Apennines

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Summary. New centric fusions (Rb 8–14 Rma) have been described in a 22-chromosome karyotype from a Mus musculus population in southern Central Italy. The diakinesis of hybrids obtained by crossing mice with different 22-chromosome complements show a ring-multivalent made up 16 metacentrics pairing arm-to-arm.

In previous papers[2,3] we reported on a population of feral house mice (Mus musculus L.) found in the Central Apennines, characterized by a 22-chromosome complement. This unusual karyotype arises from the 40-chromosome standard mouse karyotype[4] through Robertsonian fusions involving all acrocentric pairs except the smallest autosomal pair, i.e. No. 19 of the standard, and the heterochromosomes. The arrangement of the acrocentric autosomes in forming the 9 pairs of Robertsonian metacentrics has already been demonstred[5,6] by means of a Trypsin-Giemsa banding procedure.

As a consequence of an extensive field study carried out in a large area of Central Italy, mice were observed in southeastern part of the Central Apennines with a 22-chromosome karyotype, but in which the 9 Robertsonian metacentric pairs were found to be different from those previously described in the Apennine mice. These differences, although not marked, were revealed by a careful karyometric analysis. The different morphology of the chromosomes was interpreted as indicative of a different arrangement of the acrocentric arms, i.e. the acrocentric chromosomes of the standard mouse karyotype, in setting up the Robertsonian metacentrics.

The distribution area of this new 22-chromosome population of mice is shown in figure 1; it includes several mountain and hill localities of Molise, the Gargano peninsula and part of nortern Puglia. Karyological studies were carried out in 20 animals collected from 6 different villages. A laboratory imbred strain has been obtained and it is, at present, kept in our breeding station. The animals of this strain have been marked by the code CB, whereas the animals belonging to the strain resulting from the 22-chromosome mouse population previously described[2,3,5] are indicated by the code CD.

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