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Tetraploid male mosaics induced by pressure.

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Within animal species, Fankhauser (1955) showed that cell volume is typically proportional to ploidy, whereas body size is not. His rules make sense because (1) cytoplasmic mRNA and protein reflect the number of active gene copies (Osborn *et al.*, 2003), while (2) organ sizes are dictated by the diffusion ranges of morphogens and the timing of hormones (Martín-Castellanos and Edgar, 2002; Stern, 2003; Vincent and Dubois, 2002).

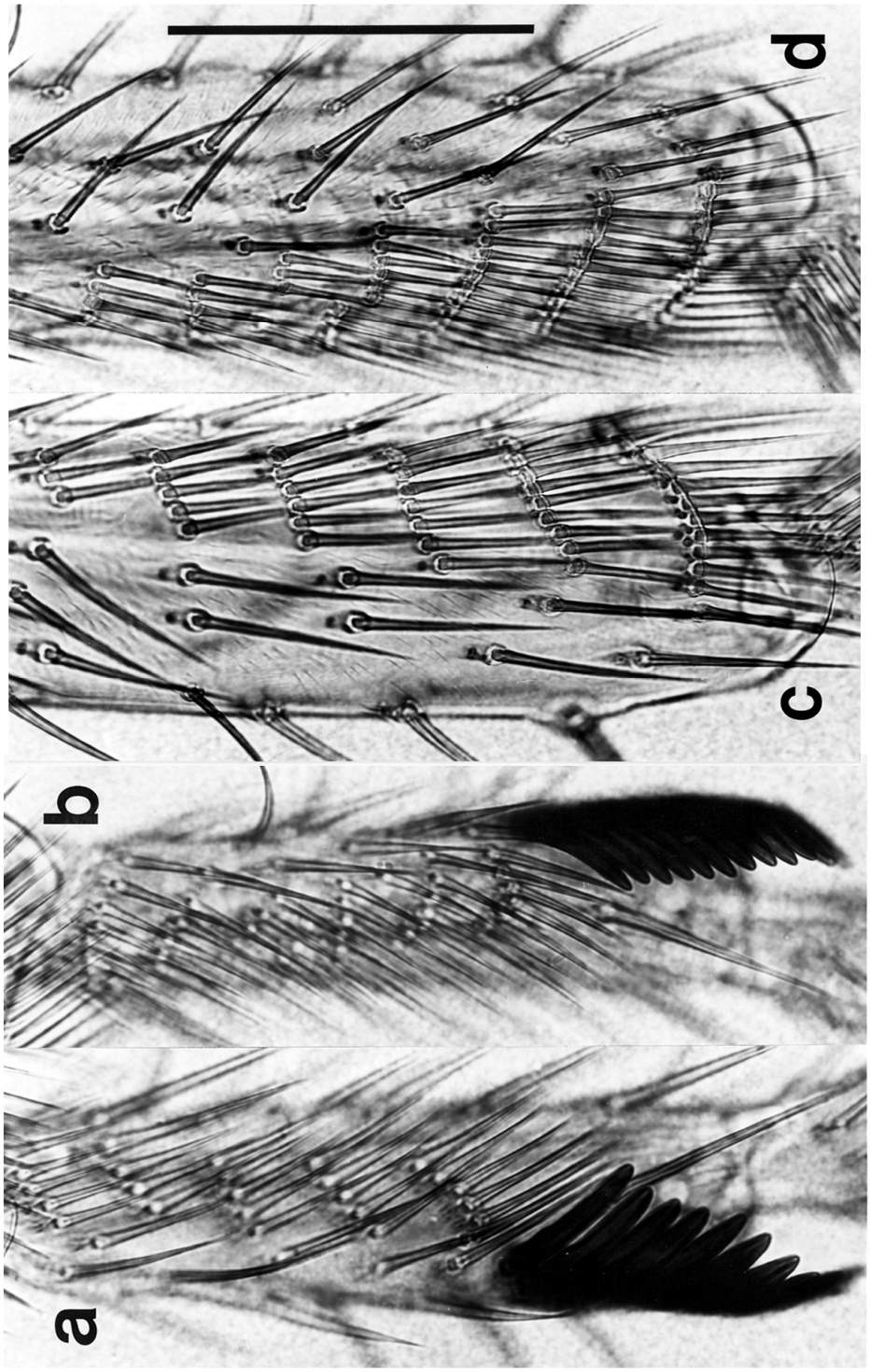

Figure 1. Left vs. right forelegs of a putative 2n/4n mosaic male (#e183). **a**, **b**. Right basitarsus with thick sex comb teeth (**a**) vs. left basitarsus with normal teeth (**b**). Bristles of the transverse rows are also thicker (and more widely spaced) in panel **a**. **c**, **d**. Right (**c**) vs. left (**d**) tibia of the same legs, showing a similar discrepancy in bristle thickness and spacing. Scale bar in **d** is 100 microns. All pictures are at the same magnification.

Wild-type *D. melanogaster* are diploid (2n). Triploid (3n) flies arise as offspring of adults that are fed colcemid (a tubulin-binding drug) during the larval period (Held, 1982), and they obey Fankhauser's rules (Held, 1979). Attempts to produce tetraploid (4n) flies by this method have failed, however, as have attempts using compound autosomes (Novitski, 1984; L. Held, unpublished). In each case, a serious obstacle is sterility.

Rare cases of 4n females have occurred as offspring of 3n females crossed with 2n males (Bridges, 1925; Morgan, 1925), but no 4n males have ever been found (Ashburner, 1989). The lack of 4n males calls into question the viability (or gender) of cells that carry 2Xs, 2Ys, and 4 sets of autosomes. My research was undertaken, in part, to investigate this issue at a tissue level.

In the present study, pressure was used to disable the spindle as a means of doubling the chromosomes somatically (Dasgupta, 1962), thus avoiding the problem of sterility. Eggs were collected on agar plates (ethanol-acetic acid) for 60 mins. at 25˚C, aged for 22 mins. at 21˚C, then submerged and exposed to 5000 pounds per square inch for 10 mins. (≈ 1 mitotic cycle). Pressure was applied by a hydraulic press (Carver) connected to a custom-made bomb chamber via a pressure transducer (Aminco). Other pressures and times were also tried but were less effective.

Age at the treatment midpoint thus ranged from 27 mins. (~cycle 3) to 87 mins. (~cycle 10) after egg laying, when the embryo has ~8 to 750 syncytial nuclei dividing in waves (Foe *et al.*, 1993). After treatment, pressure was reduced gradually (-500 psi/sec.). Control batches were submerged but not pressurized. Treated embryos were transferred to food bottles for the duration of development. Survival of pressurized embryos to the adult stage was only 8.5% (N = 200), compared with 90.5% (N = 200) for submerged controls.

Wings are easier to screen for ploidy than legs due to their flatness and uniform hairs, each of which is made by one cell. Wings were examined at 150× magnification for sparse hairs indicative of large cells. Patches of sparse hairs were found in 17/185 experimental males (9%) on one (15 cases) or both (2 cases) wings and in 12/222 experimental females (5%) on one (11 cases) or both (1 case) wings. Patches ranged in size from ~10% of one surface to virtually the entire wing (7 male and 3 female wings). No such patches were found on 108 control wings.

The legs of these 17 males were mounted between cover slips (in Faure's solution) and examined at 200× for abnormal sex combs. Three abnormal combs were found on 3 different adults. In two cases the comb had one or two teeth (bristles) that were obviously thicker.

The third case (right foreleg of male #e183) was striking. All 11 teeth in this comb were thick (Figure 1a), whereas the left comb (12 teeth) was normal (Figure 1b). The rest of the right foreleg had thick bristles as well, and they were widely spaced (Figure 1c)—indicating 4n ploidy. Indeed, the entire right 2nd and 3rd legs appeared 4n, as did the right wing (not shown). Evidently, this male came from an embryo, most of whose nuclei on the right side were dividing when pressurized.

The existence of combs with 4n-size teeth implies that 2Xs, 2Ys, and 4 sets of autosomes specifies maleness. This inference is consistent with the orthodox model of sex determination, where gender depends on the X:autosome ratio (Cline and Meyer, 1996). The sample size here is too small, however, to draw any conclusion about penetrance.

Because bristle spacing is proportional to cell diameter (Held, 1979; Stern, 2003), Fankhauser's rules imply that bristle number should decrease on polyploid legs—a correlation already documented in 3n flies (Held, 1979.). This prediction is also met in the 4n legs here. For example, rows 1-8 on the 2nd-leg basitarsi of male #e183 had the following numbers of bristles, where each ordered pair gives left (2n) vs. right (4n) data as "(L, R)": row 1 (11, 10), row 2 (10, 7), row 3 (7, 4), row 4 (5, 4), row 5 (6, 6), row 6 (7, 6), row 7 (10, 8), row 8 (13, 11).

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Sperm management in the sperm heteromorphic species, *Drosophila teissieri*.

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Apart from the species of the *D. obscura* group, in which all examined species showed a sperm heteromorphism (Joly and Lachaise, 1994; Snook, 1997), *D. teissieri* is another drosophilid species showing such a phenomenon (Joly *et al*., 1991; Lachaise and Joly, 1991). The heteromorphism in *D. teissieri* differs from the one observed in the *D. obscura* group species because the two sperm length morphs are not discrete classes but two major peaks in a wide and continuous distribution. Furthermore sperm length in *D. teissieri* is always longer than in the *D. obscura* group species (*i.e*., from 0.139 mm for *D. obscura* to 0.925 mm for *D. azteca* for the long sperm morph within the *D. obscura* group species, and 1.606 mm for the long sperm morph in *D. teissieri*; Joly *et al.,* 1989). If it now seems clear that only long sperm are fertilization competent in the *D. obscura* group species (Bressac and Hauschteck-Jungen, 1996; Snook, 1997; Snook and Karr, 1998), this question is still open for *D. teissieri*. To a better understanding of the reproductive strategy of this species, we performed a quantitative analysis of sperm production, transfer and storage at different times after mating.

Sperm production in *D. teissieri* males was determined by dissecting the two seminal vesicles of one-week-old non-mated males. The sperm mass was spread and the number of sperm determined by visual observation under a fluorescence microscope, after ethanol fixation and DAPI staining method for nucleus (Bressac and Hauschteck-Jungen, 1996). The number of sperm transferred during copulation was recorded dissecting the female genital tract just after the end of copulation of one-week-old pair of flies. In order to determine the number of stored sperm, the females were isolated just after the copulation and kept in vial with 10 ml of standard food for one, five or eight days. At the end of this period, the females were dissected and the number of sperm present in the storage organs, *i.e*. the ventral receptacle and the two spermathecae, was counted after DAPI staining.