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Rare male mating advantage has been defined as frequency-dependent male sexual fitness with the rare type of male favoured. The phenomenon has been described frequently, but it is still a subject of controversy (reviewed by Knoppin 1985). It has been suggested recently that in some cases the rare male

effect may be merely an artifact of experimental methods, for instance, when males used for the mating tests are not sampled randomly with respect to place in the storage vial (Markow 1980). The possibility that alternately harming the rare and the common strain as a consequence of marking may produce a rare male effect is discussed by Knoppin (1985). The same applies to the many statistical pitfalls in this area. It is the purpose of this paper to discuss yet another possibility to create an artificial rare male effect.

The idea is based upon a finding of Van den Berg (1985, this issue). He found a profound effect of the number of males stored per vial on subsequent male courtship behaviour, directed to a decoy. This decoy consisted of a dead male body, deprived of its own odour, and supplied with female odour. Males stored singly preceding the test were shown to be much more persistent for a number of courtship elements (orientating and wing vibrating) than males stored together in numbers of 25 per vial. In a rare male experiment males may be stored preceding the mating test according to the number in which they are needed for each desired ratio, simply because this is a practical and convenient way of experimental setup. However, in this case the males needed for the low ratio of either type turn out to be the most persistent in courting. This may create an artificial rare male effect because the more actively courting males probably also will be superior in mating.

The following findings support this idea. It is predicted by the finding of Van den Berg that males previously stored singly are superior in mating to males stored together with other males. This prediction was tested in experiments in which two males, each with a different storage history, were competing for one female. For this purpose flies of *D.melanogaster* were derived from the Groningen population. Virgin males and females were etherized once to separate the sexes, and subsequently aged for 3-4 days at 25°C. Males were either stored as single males or in numbers of 10 per vial; all females were stored in numbers of 10 per vial. The wing of the former type of male was clipped throughout the experiment for identification. One female and two males were combined in vials, and mating was observed for maximally one hour. As soon as copulation took place, the mating couple was removed, and the type of the male was identified after the experiment. In the 90 runs of this experiment 89 matings took place: 53 by males stored singly and 36 by males stored in numbers of 10, which is significantly in favour of the singly stored males ($P < 0.04$, binomial distribution, normal approximation, one-tailed test). Next a more direct test was made to decide whether the number of males stored per vial could cause bias in rare male experiments. Flies of *D.melanogaster* were derived from the Bogota population. Virgin males and females were etherized once to separate the sexes, and subsequently aged for 3-5 days at 25°C. Both sexes were stored in vials in numbers of either 1 or 9 to serve as "rare", respectively "common" flies. Throughout the experiment the wing of the rare flies was clipped for identification. Mating experiments were conducted by direct observation during one hour in slightly modified Elens-Wattiaux mating chambers (Van den Berg et al. 1984) with ten pairs of flies per run (1 male and 1 female rare, and 9 males and females common), while copulating pairs were not removed, thus allowing any individual male more than one copulation. In this experimental setup it was found that rare males are superior in mating to common males (Table 1). This can be shown to be significant with the test applied by Pruzan (1976) ($P < 0.04$).

A preliminary for the interpretation of these findings is that wing clipping does not affect mating success. A mating disadvantage, if any, of clipped males seems most likely (Robertson 1982), which is the reason that only the males stored as a single male, from which superiority in mating was hypothesized, were clipped. Also a rare male effect for wing clipping seems unlikely (Ehrman 1966).

It is concluded that it is imperative to keep in mind that differential storage conditions can bias the outcome of a rare male experiment.

Table 1. Mating success of flies stored singly, used as rare (R) flies, compared with flies stored in numbers of 9, used as common (C) flies, determined by direct observation, with 10 pairs of flies per run.

Frequency of rare type	No. of runs	Matings	♀ x ♂	R♀	C♀	R♂	C♂
		RR	RC	CR	CC		
0.10	14	3	10	16	91	13	107
						19	101

References: Ehrman, L. 1966, *Anim. Behav.* 14:332-339; Knoppin, P. 1985, *Biol. Rev.* 60:81-117; Markow, T.A. 1980, *Behav. Genet.* 10:553-556; Pruzan, A. 1976, *Evol.* 30:130-145; Robertson, H.M. 1982, *Anim. Behav.* 30:1105-1117; Van den Berg, M.J. et al. 1984, *Behav. Genet.* 14:45-61.