Pregnancy blocking in the vole, *Microtus agrestis*

I. Effect of the social environment

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Summary. Pregnancy failed in a high proportion of newly mated *Microtus agrestis* females when they were exposed to a strange male between 48 and 72 hr after mating with a stud male. This effect of the strange male was testosterone dependent. Direct contact with the strange male was normally necessary, and even a single barrier of wire mesh between the female and strange male prevented the male from exerting his pregnancy-blocking effect. The results suggest that the stimuli mediating pregnancy blocking in the vole may differ from those operative in the mouse.

Introduction

The failure of pregnancy in a high proportion of newly mated females following exposure to a 'strange' male, i.e. a male other than the stud, was first observed in the mouse (Bruce, 1959). More detailed investigations indicated that pheromones in the male urine mediated this effect (Bruce, 1960a; Parkes & Bruce, 1962; Dominic, 1966). The characteristics of the 'Bruce effect' suggest that the immediate cause of the block to pregnancy is a failure of CL function, which in turn is responsible for a failure of implantation (Bruce, 1960a; Parkes & Bruce, 1962; Bronson & Eleftheriou, 1963).

The pregnancy-blocking effect of a strange male has also been demonstrated in *Peromyscus maniculatus* (Eleftheriou, Bronson & Zarrow, 1962), *Microtus agrestis* (Clulow & Clarke, 1968), and *M. pennsylvanicus* (Clulow & Langford, 1971). In addition, there is suggestive evidence for the phenomenon in *Clethrionomys glareolus* (Clarke & Clulow, 1973). Little is known, however, of the nature of the stimuli causing the block to pregnancy in these species. This paper reports studies undertaken to extend the observations in the vole, *Microtus agrestis*.

Materials and Methods

Laboratory-bred voles were used in all experiments. The main breeding colony was established in 1959 and was a closed colony until 1972. Animals from this colony are referred to as 'laboratory-type' (LT). In 1971, a separate, subsidiary breeding colony was established, composed of animals caught in the wild and their first generation offspring; animals from this colony are referred to as 'wild-type' (WT). In 1972, WT animals were introduced into the main breeding colony, and animals from this mixed colony are referred to as 'mixed-type' (MT). All animals were maintained as described by Breed (1969). Vaginal smears were taken daily with a platinum wire loop and classified as described by Milligan (1974).

Mature females were individually housed in wire-topped plastic cages (29 × 19 × 10 cm). Two days later, a fertile male (the stud) was introduced into each female's cage between 08.30 and 10.30 hours. Twenty-four hours after pairing, each female was examined for evidence of mating (spermatozoa in the vaginal smear, or a copulatory plug). Animals that had mated were left together for a further 24 hr. At 48 hr *p.c.* each female was transferred to a clean cage and exposed to an experimental treatment for 24 hr (see Table 1). The details of the treatments are as follows. *Treatment A* (all Exps): the stud male was re-introduced into the female's cage. *Treatment B* (all Exps): a 'strange' male was introduced into the female's cage. *Exp. 1C*: three strange males were housed on a wire-mesh platform

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which served as the roof of the female's cage. *Exp. 4C*: a strange male was introduced into the female's cage, but was separated from her by a single barrier of wire mesh (0.8×0.8 cm mesh). *Exp. 4D*: as for *Exp. 4C*, but two barriers of mesh, 2.5 cm apart, separated the male from the female. *Exp. 4E*: urine and faeces from 3 strange males were delivered into the female's cage by a glass funnel. The males lived on a wire mesh floor 30 cm above the female's cage. *Exp. 5C*: the female was housed in a cage which had been soiled by 3 or 4 males during the preceding 24 hr. *Exp. 5D*: as for *Exp. 5C*, but the cage had been soiled by 3 or 4 females. *Exp. 6C*: as Treatment B, but the strange male had been castrated at least 3 weeks earlier. *Exp. 6D*: as *Exp. 6C*, but a 25-mg testosterone pellet had been implanted s.c. in the castrated male at least 2 weeks earlier.

After the treatment period, each female was transferred to a clean cage where she remained until the completion of the experiment.

Females in *Exp. 1* were not killed until at least 3 weeks after the stud mating; litters born to the females were recorded. All other females were killed within 6 to 8 days of the stud mating. Pregnant females possessed large vascular CL and uterine implantation sites, and exhibited thin or leucocytic vaginal smears from shortly after mating until they were killed. Females were judged to have had their pregnancies blocked if they exhibited a return of vaginal smears containing predominantly cornified cells within 7 days of the stud mating, and if, in addition, they lacked implantations and possessed either no CL or only degenerating CL (Milligan, 1976).

Females that were pseudopregnant when killed, and females in Treatment B that mated with both the stud male and the strange male, were excluded (these constituted less than 5% of the total animals used). Results were analysed with χ^2 tests.

Results

As shown in Table 1, in all voles except those in *Exp. 5*, caging newly mated females with an intact strange male (Treatment B) rather than the stud male (Treatment A) produced a significant pregnancy-blocking effect. Although the % of pregnant females in *Exp. 5B* was significantly lower than in *Exp. 5C* ($\chi^2 = 9.37$; $P < 0.01$) and *Exp. 5D* ($\chi^2 = 12.4$; $P < 0.001$), the difference between *Exps 5A* and *5B* was not significant ($\chi^2 = 2.48$; $P > 0.05$). The only other treatment involving intact strange males that resulted in a significant blocking effect was caging 3 males directly above the female (*Exp. 1C*; $\chi^2 = 6.78$; $P < 0.01$). Castrated males failed to block pregnancy (*Exp. 6C*), but testosterone-treated castrates exerted a significant blocking effect (*Exp. 6D*; $\chi^2 = 7.26$; $P < 0.01$).

Discussion

The results from these experiments confirm the report of Clulow & Clarke (1968) that caging newly mated *Microtus agrestis* females directly with strange males causes a failure of pregnancy in a high proportion of animals. This effect occurred in parous and nulliparous LT females (*Exps 1, 3* and *5*) and in WT females (*Exp. 2*).

Experiments 1, 4 and 5 were designed to determine the nature of the effective stimuli provided by the strange male. Only two treatments, however, had any significant pregnancy blocking effect: caging the female directly with the strange male (Treatment B of *Exps 1* to *4*) and caging the female directly below strange males (*Exp. 1C*). Separating the female from a strange male by a single or double barrier of wire mesh (*Exps 4C* and *4D*), exposing the female to the fresh urine and faeces of strange males (*Exp. 4E*) and caging the female in cages soiled by males or females (*Exps 5C* and *5D*), all failed to produce any significant reduction in pregnancy rate. Pregnancy is readily blocked in newly mated female mice on exposure to strange males in situations similar to those that were unsuccessful in the present study (Bruce, 1959, 1960a, b; Parkes & Bruce, 1962; Chipman & Fox, 1966b; Dominic, 1966). Pheromones in the urine of the male are believed to be the effective stimuli causing pregnancy block in the mouse, and pregnancy and pseudopregnancy are blocked by the

application of urine from the strange male to the external nares of newly mated females (Dominic, 1964, 1966). The close contact needed between the female and strange male to achieve the pregnancy-blocking effect in *M. agrestis* suggests that air-bound olfactory stimuli may not be the sole mediators of the effect in this species.

Table 1. Pregnancy blocking in newly mated female voles in various experimental situations

		No. of females			
		Total	Pregnant	Not pregnant	% Pregnant
Experiment 1 (LT nulliparous females)	A. Stud male	20	17	3	85
	B. Strange male	17	3	14	18**
	C. Three strange males above female	18	7	11	39*
Experiment 2 (WT nulliparous females)	A. Stud male	13	12	1	92
	B. Strange male	14	4	10	29*
Experiment 3 (LT parous females)	A. Stud male	12	12	0	100
	B. Strange male	9	3	6	33*
Experiment 4 (MT nulliparous females)	A. Stud male	15	14	1	93
	B. Strange male	15	3	12	20**
	C. Strange male behind single barrier	14	12	2	86
	D. Strange male behind double barrier	14	13	1	93
	E. Urine and faeces from three strange males	14	9	5	64
Experiment 5 (LT nulliparous females)	A. Stud male	11	7	4	64
	B. Strange male	10	2	8	20
	C. Housed in cage soiled by males	11	10	1	91
	D. Housed in cage soiled by females	9	9	0	100
Experiment 6 (MT nulliparous females)	A. Stud male	12	12	0	100
	B. Strange male	12	5	7	42*
	C. Castrated male	12	11	1	92
	D. Testosterone-treated castrated male	12	5	7	42*

† See Text for details.

* Significantly different from Treatment A, $P < 0.01$.

** Significantly different from Treatment A, $P < 0.001$.

It is possible that pheromones transferred by tactile contact may be involved (Weir, 1973; Milligan, 1975b). The strange male failed to exert any significant pregnancy blocking effect, however, when tactile contact between the animals was possible through a single barrier of wire mesh (Exp. 4C), and the phenomenon in the vole may be associated, at least in part, with a behavioural component. When the strange male was caged directly with the newly mated female, he often attempted to court her, even though she was unreceptive. The above suggestion does not, however, explain the observation that caging females immediately below strange males had a pregnancy blocking effect (Exp. 1C). The intensity of 'strange male' stimulation provided by the 3 males in close proximity to the female, may have been sufficient stimulus, or the physical rather than social conditions of this treatment may have caused the effect, as changes in the physical environment can cause pregnancy

failure in mice (Chipman & Fox, 1966a) and deermice (Eleftheriou *et al.*, 1962). The results from Exp. 6 indicated that, whatever the nature of the effective stimuli from the strange male, they are testosterone dependent. Further work is planned to determine the nature of these stimuli.

The significance of the pregnancy-blocking effect to animals in the wild is not known, but the ability to induce oestrus in a female at the expense of another male's insemination is likely to possess a considerable selective advantage (Whitten & Bronson, 1970). The nature of the phenomenon also suggests that it may have a role in controlling population density (Chipman, Holt & Fox, 1966; Clulow & Clarke, 1968; Whitten & Bronson, 1970), and Mallory (1972) has suggested that pregnancy blocking may be involved in the population fluctuations of microtine rodents. A number of variables, however, may affect the importance of pregnancy blocking to animal populations in the wild: in mice, laboratory experiments indicate that some strains are more susceptible than others (Chapman & Whitten, 1969) and that blocking effectiveness may be reduced by grouping the females (Bruce, 1963), pre-exposing the females to many males, or the continued presence of the stud male (Parkes & Bruce, 1962).

There has been no satisfactory demonstration, however, that pregnancy blocking actually occurs in wild rodents. It has been suggested that the observations of successive generations of CL in the ovaries of such microtine species as *Clethrionomys glareolus*, *C. rutilus*, *Microtus pennsylvanicus*, and *M. agrestis* may reflect successive pregnancies which have been blocked (Clarke & Clulow, 1973; Clulow & Mallory, 1974). Although the different generations of CL must reflect a failure of normal CL function followed by another ovulation, recent observations suggest that it may be unnecessary to invoke the phenomenon of pregnancy blockage to account for the CL failure. In *M. agrestis*, short-lived CL may also result from ovulations induced either by limited mating (Milligan, 1975a) or by more remote male contact (Milligan, 1974). If such CL also occur in other microtine species, it may be difficult to obtain convincing evidence for pregnancy blocking in wild animals on the basis of ovarian histology alone.

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