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Studies of the Male-Originating Pheromones Involved in the Whitten Effect and Bruce Effect in Mice

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ABSTRACT

Experiments were designed to elucidate the mode of transmission of the male-originating pheromones involved in the induction of estrus (the Whitten effect) and in implantation failure (the Bruce effect) in mice. The Whitten effect was induced in unisexually grouped females by exposure to corralled males, and also by corralled males housed within a perforated cage (which prevented physical contact of the females with the male-originating pheromone). The results suggest that the pheromone involved in the Whitten effect is volatile (airborne).

Implantation failure occurred in a significantly high proportion of newly inseminated females when they were individually confined in corrals and housed below corralled alien males. By contrast, implantation failure was significantly reduced when corralled females were housed above corralled alien males. The results indicate that the male-originating pheromone involved in the Bruce effect is nonvolatile and acts on the females through contact.

It is suggested that the pheromone involved in the Whitten effect is distinct from the one involved in the Bruce effect.

INTRODUCTION

It is well established that certain reproductive processes in female mice are profoundly influenced by the presence by conspecific males (cf., Whitten, 1966; Bronson, 1971, 1979; Dominic, 1978; Aron, 1979). The male induction and synchronization of estrus among unisexually grouped females (the Whitten effect), and the male-induced failure of implantation in newly mated females (the Bruce effect) are two well-known examples of male pheromonal effects in mice (cf., Whitten, 1966).

The pheromone involved in the Whitten effect is excreted in the urine of intact adult males (Marsden and Bronson, 1964; Bruce, 1965), and its production is androgen dependent (Bruce, 1965; Dominic, 1968). Likewise, the male-originating pheromone causing the Bruce effect is also voided in the urine of intact adult males (Dominic, 1966), and its production is androgen dependent (Bruce, 1965; Dominic, 1965).

It is generally believed that the male-originating pheromone(s) involved in the Whitten effect and the Bruce effect is olfactory (cf. Whitten, 1966; Dominic, 1978). Whitten et al. (1968) provided evidence suggesting that the male-originating pheromone causing induction of estrus is volatile and airborne. However, the report (Marchlewska-Koj, 1977) that a single high molecular weight protein fraction from male mouse urine is capable of blocking ovoimplantation in newly inseminated females, and shortening the estrous cycle in individually housed females, suggests that male mice produce only one pheromone and that it is unlikely to be volatile (airborne). The present investigations were designed to evaluate the role of physical contact in the mediation of the pheromonal stimuli involved in the Whitten effect and Bruce effect.

MATERIALS AND METHODS

All animals, except the alien males employed in Experiment 2, were laboratory bred, albinos of the Parkes (P) strain. The alien males used for exposure to newly inseminated females belonged to the wild strain. The animals were housed under standard laboratory conditions and maintained on pelleted food (Hindustan Lever Ltd., Ghaziabad, India) and water ad libitum. Two experiments were designed to evaluate the role of contact in the pheromonal transmission in the Whitten effect (Experiment 1) and the Bruce effect (Experiment 2).

Experiment 1

Thirty regularly cycling, 10- to 12-week-old, virgin P females were unisexually housed in a colony

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cage, 68 X 68 X 14 cm (Fig. 1a) for 28 days (Days 1 to 28), and then exposed to two adult P males for 21 days (Days 29 to 49). During exposure the males were individually confined in wire mesh corrals, 15 X 12 X 9 cm, and placed in the center of the cage housing the females (Fig. 1b). The males with the corrals were then removed and the females again remained unisexually grouped (Fig. 1c) for the next 21 days (Days 50 to 70). The females were then again exposed to two adult P males for 21 days (Days 71 to 91). The males were individually corralled as before, and the two corrals were placed in the center of a metal cage, 40 X 15 X 10 cm, with perforated walls and a solid bottom. The corrals were positioned in the perforated cage in such a way that they were separated from the walls of the perforated cage by about 1.5 cm. The perforated cage containing the corralled males was then placed in the center of the colony cage housing the females (Fig. 1d). This arrangement prevented the physical contact of the females with the males or male urine/excreta.

Vaginal smears were examined daily from all females except on the first 7 days after unisexual grouping (Days 1 to 7). Estrous cycles were classified as normal (4–6 days) and prolonged (7 days or more) (cf., Gangrade and Dominic, 1983).

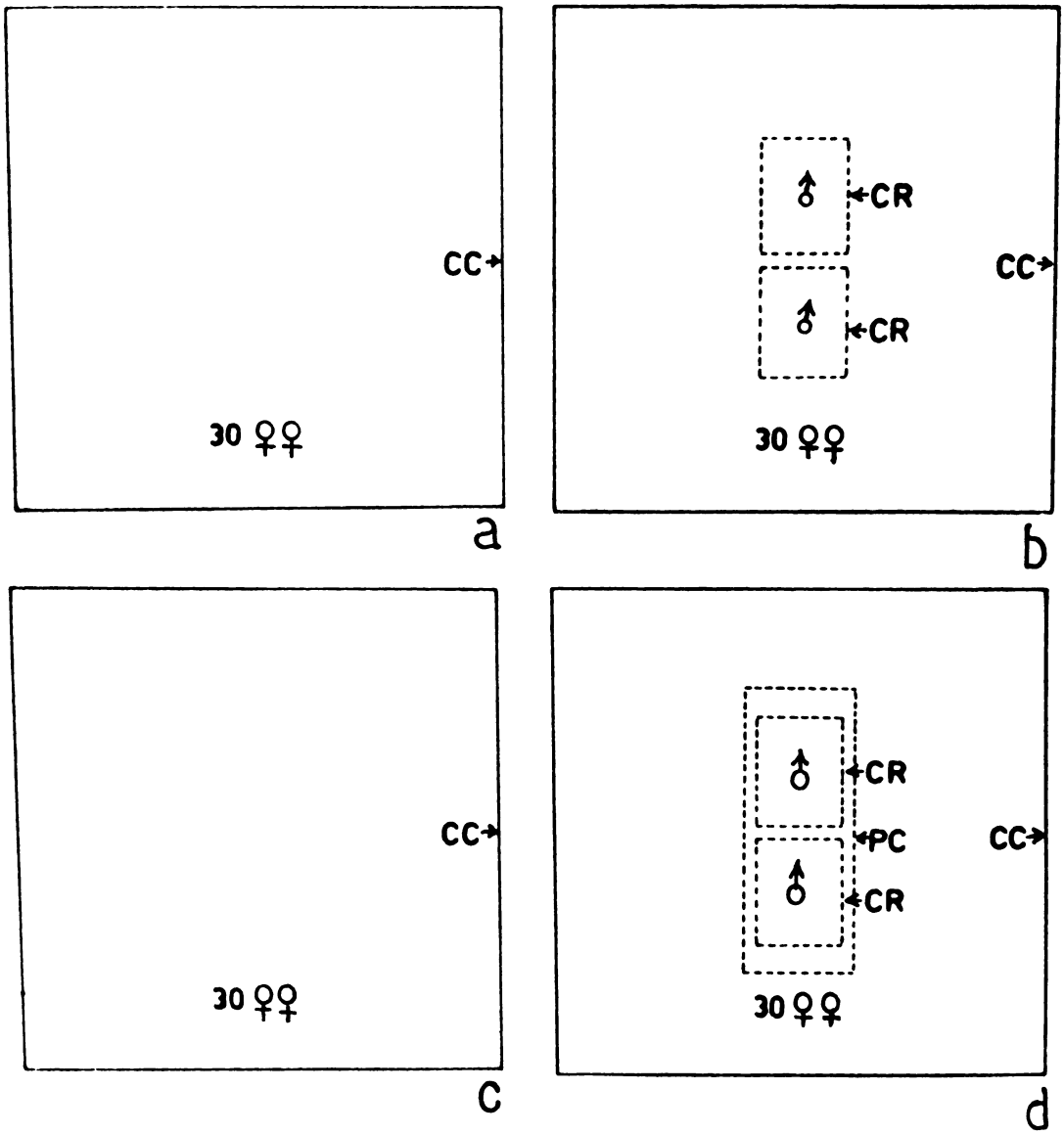

FIG. 1. Schematic representation of the setup used for evaluation of the role of contact in the Whitten effect (for details see the text). *CC*=Colony cage, *CR*=corral and *PC*=perforated cage.

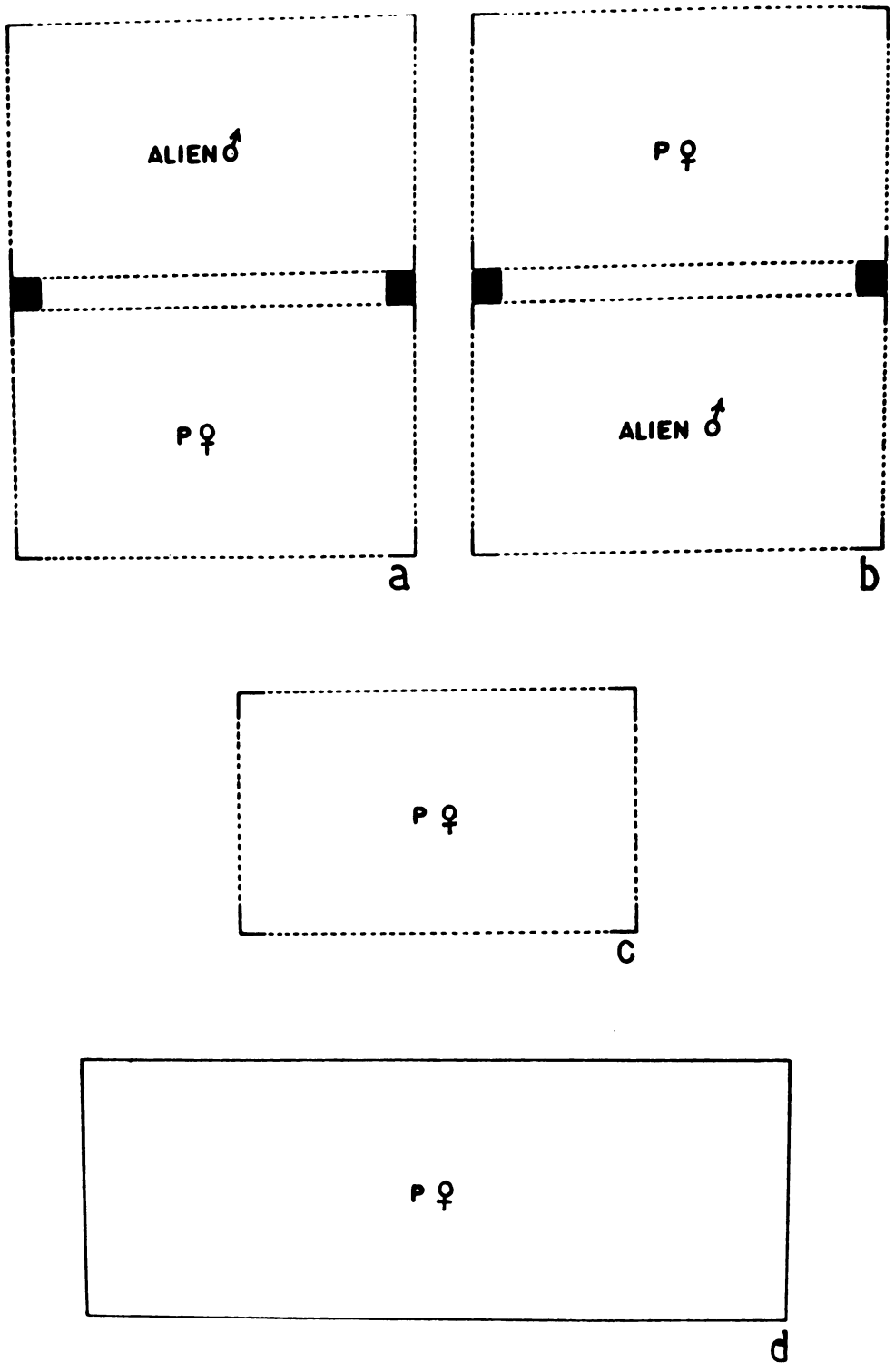

FIG. 2. Schematic representation of the setup used for evaluation of the role of contact in the Bruce effect (for details see the text).

Experiment 2

Ten-week-old, virgin P females were paired with P males. After finding the vaginal plug (Day 0), the females were separated from the stud males and housed individually either in wire mesh corrals, 15 X 12 X 9 cm (Groups I, II, and III) or in metal cages, 40 X 15 X 10 cm (Group IV). Twenty four hours later (Day 1 postcoitum) they were treated as follows:

Group I: The newly inseminated P female and the alien (wild) male were individually housed in corrals. The corral containing the alien male was positioned over the corral containing the newly inseminated female (supported by four square blocks) in such a way that the two corrals were separated by a space of 1.0 cm (Fig. 2a). The female and the alien male were housed like this for 3 days (from 1000 h on Day 1 to 1000 h on Day 4). The alien male with the corral was then removed and the female remained in her corral until Day 7.

Group II: The corral containing the newly inseminated female was placed above the corral housing in the alien male, separated by a space of 1.0 cm (Fig. 2b) as in the previous group. The duration of exposure to alien males and other housing conditions were similar to those provided for animals in Group I.

Group III: Females were individually housed in corrals (Fig. 2c) and left undisturbed after separation from the stud males.

Group IV: Females were individually housed in metal cages, 40 X 15 X 10 cm (Fig. 2d), and left undisturbed after separation from the stud males.

Vaginal smears were examined daily from all females up to Day 7 postcoitum and a return of vaginal cornification within this period was taken as the indication of implantation failure (cf., Bruce, 1960; Dominic, 1966). Females which failed to show vaginal cornification were killed on Day 7 to confirm pregnancy; those without implanted fetuses were presumed to be pseudopregnant.

Statistical Analysis

Data were analyzed by Student's t test and z test for proportions (Bruning and Kintz, 1977).

RESULTS

Experiment 1 (Tables 1 and 2)

Unisexual grouping of female mice induced extension of diestrus, resulting in the incidence of prolonged cycles (Table 1). Other irregularities like "split" estrus (cornified cells on alternate days with leukocytes on intervening days), "attempted" estrus (incomplete cornification), and prolonged vaginal cornification were also seen in grouped females. Exposure to corralled males induced estrus in the majority of grouped females within 7 days, with a peak (35.7%) on the third day (Table 2). Grouped females in the presence of corralled males exhibited short and normal cycles. Other irregularities like "split" estrus, "attempted" estrus and prolonged vaginal cornification were

TABLE 1. Cycle history of unisexually grouped females.

Group and treatment	Mean frequency of estrous cycle ± SEM[a]	Proportion and (%) of	
		Normal cycles[b]	Prolonged cycles
A. Unisexual grouping (Days 7 to 28)	0.87 ± 0.12	4/26 (15.4)	22/26 (84.6)
B. Unisexual grouping + exposure to corralled males (Days 29 to 49)	2.83 ± 0.11	81/85 (95.3)	4/85 (4.7)
C. Unisexual grouping (Days 50 to 70)	0.87 ± 0.15	10/26 (38.5)	16/26 (61.5)
D. Unisexual grouping + exposure to corralled males housed within a perforated cage (Days 71 to 91)	1.83 ± 0.11	38/55 (69.1)	17/55 (30.9)

[a,b]Significance of group differences: [a]*A* vs. *B*, *D*, $P<0.001$; *A* vs. *C*, NS; *B* vs. *C*, *D*, $P<0.001$; *C* vs. *D*, $P<0.001$.

[b]*A* vs. *B*, $P<0.001$; *C* vs. *D*, $P<0.001$; *B* vs. *D*, $P<0.001$; *A* vs. *C*, NS.

TABLE 2. Return of estrus in unisexually grouped females following male exposure.

Group and treatment	Number and (%) of females returning to estrus on days after exposure							Total number of females returning to estrus within 7 days of male exposure
	Day: 1	2	3[a]	4	5	6	7	
B. Exposure to corralled males	4 (14.3)	4 (14.3)	10 (35.7)	5 (17.8)	5 (17.8)	0 ---	0 ---	28
D. Exposure to corralled males housed within a perforated cage	2 (8.0)	3 (12.0)	8 (32.0)	7 (28.0)	4 (16.0)	1 (4.0)	0 ---	25

[a]Significance of group difference: *B* vs. *D*, NS.

also not seen when the males were present. Removal of corralled males again induced prolonged cycles and other irregularities in the females. Exposure to corralled males housed within a perforated cage also induced estrus in the majority of grouped females within 7 days with a peak (32.0%) on the third day (Table 2). Females also exhibited shorter and more regular estrous cycles in the presence of corralled males as compared to the period when corralled males were housed within a perforated cage (Table 1).

Experiment 2 (Tables 3 and 4)

A significantly higher proportion (48/54) of newly inseminated females housed below the corralled alien males (Group 1) exhibited failure of implantation as compared to the newly inseminated females housed above the corralled alien males (Group II) (Table 3). The females in which implantation failed exhibited a peak return of estrus on Day 4 (Table 4). The proportion of females showing implantation failure in Group II was not significantly different from the spontaneous failure of pregnancy seen in undisturbed females (Groups III and IV).

DISCUSSION

It is well documented (cf., Whitten, 1966) that unisexual housing of regularly cycling female mice induces disruption of the estrous cycle, and exposure of such females to conspecific males induces a new estrous cycle in the majority of individuals resulting in the synchronization of estrus 3 days later. Moreover, female mice in the presence of males exhibit shorter and more regular cycles than in their absence (cf., Whitten, 1966). Whitten et al. (1968) provided evidence suggesting that the male-originating pheromone involved in the induction of estrus (the Whitten effect) is volatile and airborne. The report (Monder et al., 1978) that a volatile lipid portion of the male mouse urine is capable of shortening the estrous cycle is in agreement with this view. However, the retention of the ability to induce the Whitten effect in a protein fraction from male mouse urine (Marchlewska-Koj, 1977) suggests that the operative pheromone is unlikely to be volatile and airborne. Our studies have confirmed earlier reports of the ability of confined (corralled) males to induce the Whitten effect and maintain regular estrous cycles in grouped females. Exposure to corralled males

TABLE 3. Implantation failure in newly inseminated females following alien male exposure.

Group and treatment	Number of females	Number and (%) of females		
		With implantation failure[a]	Remaining pregnant	Remaining pseudopregnant
I. Corralled female housed below corralled alien male	54	48 (88.9)	6 (11.1)	0
II. Corralled female housed above corralled alien male	47	11 (23.4)	33 (70.2)	3 (6.4)
III. Corralled female left undisturbed	25	2 (8.0)	22 (88.0)	1 (4.0)
IV. Female left undisturbed in the cage	26	2 (7.7)	23 (88.5)	1 (3.8)

[a]Significance of group difference: *I* vs. *II*, *III*, $P<0.001$; *II* vs. *III*, NS; *III* vs. *IV*, NS.

housed within a perforated cage (which prevented direct contact of the female with male urine and excreta but exposed them to the airborne olfactory cues from males) also induced the Whitten effect and maintained regular cycles in grouped females, though contact with male urine/excreta was more effective in this regard. It is possible that the male-originating pheromone involved in the Whitten effect is a lowly volatile substance. This may account for the significant decrease in the frequency of estrous cycles and the proportion of normal cycles in females when exposed to corralled males housed within a perforated cage, as compared to when they were exposed to corralled males. Our findings are in general agreement with the view (Whitten et al., 1968) that the male pheromone involved in the Whitten effect is volatile and airborne and acts on the females through olfactory pathways. Relevant to the present discussion is the report (Gangrade and Dominic, 1983) of the failure of males to abolish estrous cycle irregularities in individually housed female mice made anosmic by intranasal irrigation with $ZnSO_4$, obviously due to the inability to perceive the male-originating pheromone.

The urinary pheromone involved in the Bruce effect is generally believed to be volatile (airborne) (cf., Parkes and Bruce, 1961; Dominic, 1966; Hoppe, 1975). The pheromone is reported to be present in the volatile lipid portion of male urine (Monder et al., 1978). The incidence of the Bruce effect in a high proportion of females housed below the corralled alien males as observed in the present study

TABLE 4. Time relations in the return of estrus in alien male-exposed females.

Group and treatment	Proportion and (%) of females with implantation failure	Number of females returning to estrus on days postcoitum					Females returning to estrus within Day 4 postcoitum (%)
		Day: 3	4	5	6	7	
I. Corralled female housed below corralled alien male	48/54 (88.9)	10	29	4	2	3	81.3
II. Corralled female housed above corralled alien male	11/47 (23.4)	2	3	2	2	2	45.5
III. Corralled female left undisturbed	2/25 (8.00)	0	1	0	0	1	50.0
IV. Female left undisturbed in the cage	2/26 (7.7)	0	1	0	1	0	50.0

is in agreement with the findings of Dominic (1966) who reported implantation failure in a high proportion of females exposed to urine from alien males housed above their cages. However, the present results indicate that implantation failure is significantly reduced when corralled, newly inseminated females are housed above the corralled alien males. In fact, the percentage of implantation failure in such females was not significantly different from the percentage of implantation failure in undisturbed females, whether confined in a corral or freely housed in a cage. Our findings, therefore, provide strong circumstantial evidence suggesting that the pheromone involved in the Bruce effect is nonvolatile and acts through contact. The report of a significant decrease in implantation failure in newly inseminated females housed with alien males, but prevented from having direct contact with the latter (Rajendren and Dominic, 1983), and of the presence of a high implantation-blocking activity in a protein fraction of the male mouse urine (Marchlewska-Koj, 1977), also suggests the nonvolatile nature of the pheromone inducing the Bruce effect. The incidence of implantation failure in females housed with confined alien males as reported earlier (Bruce, 1960; Dominic, 1966) is probably due to the direct contact of the females with the male-originating pheromone through the mesh of the corral. Likewise, in the investigations of Monder et al. (1978) the possibility of physical contact of the newly inseminated female with the lipid extract of male urine cannot be completely ruled out (cf., Johns, 1980).

The pheromones involved in the Whitten effect and the Bruce effect are present in the urine of the intact males, are androgen dependent, and presumably bring about their effects by suppressing prolactin secretion and stimulating gonadotropic activity in females; furthermore, the external effects observed, viz. induction of estrus, is the same for both pheromones (cf., Bronson, 1974; Dominic, 1978). Marchlewska-Koj (1977) reported that male urinary proteins are capable of inducing the Bruce effect and shortening the estrous cycle in females. Hence, it is suggested that the male mouse produces only one pheromone which can evoke different effects depending upon the physiological state of the female (Bronson, 1971; Marchlewska-Koj, 1977; Dominic, 1978; Whitten and Champlin, 1978). However, the present investigations suggest that the pheromone involved in the Whitten effect is volatile (airborne) and the one involved in the Bruce effect is nonvolatile and acts through contact. Hence, it appears that the male-originating pheromone causing the Whitten effect is distinct from the pheromone causing the Bruce effect.

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