Behaviour of Two-sex Groups of Mice at 4, 21 and 33°C

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Abstract
Groups of mice comprising two males only, two males plus two females, two males plus six females, or two males plus 14 females were housed in pens at 4, 21 or 33°C. The pens were partitioned into two territories connected by a small hole and each subdivision was equipped with two box shelters on either side of an open area containing food, water and woodwool.

At all group sizes, the mice at 4 and 21°C sought shelter more frequently than those at 33°C. In addition, those at low temperatures tended to crowd into one of the nesting boxes, while those at 33°C distributed themselves fairly evenly between the four shelters. At all temperatures the males shared the whole of the territory available to them, but at 33°C they were found in the same shelter at the same time only once out of 40 occasions; those at 4 and 21°C were found together on more than 25 occasions. More woodwool and more urination sites were found in the shelters at 4 and 21 than at 33°C, but temperature had little effect on the amounts of food and faeces in the shelters.

Hence, it appears that contact between the individual and its companions in male–female groups is, as in all-female groups, inversely related to temperature. It also seems that contact between males in such groups is markedly reduced at high temperatures.

Introduction
When males are released into groups of 15 females housed at 4, 21 and 33°C, more females become pregnant at 33°C than at the two lower temperatures (Pennycuik 1972). One noticeable difference between groups of females at 33°C and those at 4 and 21°C is that those at the higher temperature have less contact with one another, and with the excretions of their companions, than those at the two lower temperatures. This observation led to the suggestion that the differences in reproductive productivity in grouped animals housed at 4, 21 and 33°C might be due to differences in the degree of contact the animals had with female primer pheromones produced by their companions (Pennycuik 1973).

Crowcroft (1966) observed that groups of male mice behaved differently from groups of females when housed in pens with several shelters: females crowded into one or two nesting boxes, but males occupied separate shelters unless the weather was very cold. If the presence of males altered the behaviour of grouped females, the suggestion that productivity was dependent upon the amount of contact with female primer pheromones might not hold. This paper reports a preliminary investigation into the behaviour of groups composed of two sexes.

Materials and Methods
The mice were exposed to temperatures of 4, 21 and 33°C. The coldroom, air-conditioned laboratory and chicken incubator used for these experiments are described elsewhere (Pennycuik 1972). The pelleted diet and the nesting material are also described elsewhere (Pennycuik 1972, 1973).
For the purposes of this experiment the pens used previously (floor area 0.25 m²) were modified so that the males in the group could, if they wished, establish two territories. Each pen was divided lengthwise by a partition pierced by one mouse-sized hole. Each of the two compartments thus formed was equipped with two nesting boxes (each 1600 cm³) placed at either end of the pen, and with a water bottle. Food and nesting material were supplied ad libitum in the open area between the boxes.

Fig. 1. Numbers of mice, amounts of woodwool, food and faeces, and urine scores in the shelters at temperatures of 4 (■), 21 (○) and 33°C (△). Mouse numbers were means of the 10 observations made during the week for each group size; weights of woodwool, food and faeces were the amounts accumulated during one week; and urine scores were assessed by assigning a wetness score (1-6) to each site at each observation time, summing the scores for each box and summing the scores for all boxes for one week.
The mice were from a random breeding stock formed by crossing mice from our wild colony with a laboratory stock (R70). Each pair of males were brothers of about equal weight, which had been housed in the same cage since weaning. These were vasectomized 2 weeks before the experiment was started. The females were intact.

The procedure followed was a modified version of that used previously (Pennycuik 1972). One pen was introduced into each of the three environments and allowed to remain there throughout the experiment. On day 1 of week 1, two males were introduced into each pen and allowed to settle down for 8 h. On the afternoon of day 1 each pen was examined through the wire mesh lid, and a note was made of the number of animals outside the nesting boxes. The lids of the pens were then removed and each nesting box was examined in turn. Records were kept of the number and identity of the mice in the box, of the presence or absence of a nest and of the presence and extent of urination sites (these were graded from 1 to 6 depending on the extent and wetness of the site). These examinations were repeated on nine occasions throughout week 1. At the end of the week the contents of the boxes (but not the alleys) were removed and sorted into woodwool, uneaten pellets, food crumbs and faeces. These were weighed after they had been dried at 90°C for 48 h.

On day 1 of week 2, two females were put into each pen, increasing group size to four, and the observations described above were repeated. At the beginning of weeks 3 and 4, four and eight additional females respectively were added to the existing population.

The experimental plan for each temperature was therefore as follows:

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>2♂</td>
<td>2♂, 2♀</td>
<td>2♂, 6♀</td>
<td>2♂, 14♀</td>
</tr>
</tbody>
</table>

Results

Like all-female groups, the groups of mixed sex at 33°C were found under shelter less frequently than those at 4 and 21°C, and they accumulated less woodwool and urine in their shelters than those at lower temperatures (Figs 1a, b, e). Unlike that of the all-female groups, the accumulation of food and faeces within the shelters was rather similar at all temperatures (Figs 1c, d). This was because the mixed-sex groups at the two lower temperatures carried less food to their shelters than did the all-female groups [e.g. at 4°C, four females hoarded 400 g of food in a week (Pennycuik 1973) but two males and two females hoarded only 6 g]. Because faeces tends to accumulate at sites where mice eat (Pennycuik 1973), the amount of excretory matter accumulating in the boxes was also reduced in these male-female groups.

Table 1. Number of mice in each box on the final day of observations when group size was four (two males and two females) in pens maintained at 4, 21 and 33°C, together with the urine scores on the final day of observations and the weights of woodwool, food and faeces that had accumulated during 1 week

<table>
<thead>
<tr>
<th>Measurement</th>
<th>4°C, box no.:</th>
<th>21°C, box no.:</th>
<th>33°C, box no.:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
</tr>
<tr>
<td>No. of mice in box</td>
<td>4  0  0  0</td>
<td>0  0  4  0</td>
<td>1  2  1  0</td>
</tr>
<tr>
<td>Urine score</td>
<td>3  5  5  5</td>
<td>5  2  0  3</td>
<td>3  2  1  2</td>
</tr>
<tr>
<td>Woodwool (g)</td>
<td>13·1 0·1 0·2 8-5 0·1</td>
<td>0·1 0·2 8·5 0·1</td>
<td>0·1 0·1 0·3 0·3</td>
</tr>
<tr>
<td>Food (g)</td>
<td>4·2 0 0 1·3</td>
<td>0 1·7 1·3 1·3</td>
<td>1·2 3·7 4·0 2·4</td>
</tr>
<tr>
<td>Faeces (g)</td>
<td>1·0 1·8 7·1 2·3</td>
<td>1·1 1·1 0·6 0·8</td>
<td>2·7 3·2 2·5 2·8</td>
</tr>
</tbody>
</table>

Like all-female groups, the groups of mixed sex at 33°C used all four shelters almost equally. At 4 and 21°C the mice used different shelters for different purposes and the animals were usually grouped together in only one box. Table 1 illustrates the differences in behaviour between the groups of four (two males and two females) at the three experimental temperatures. At 4 and 21°C all the mice congregated in the box set aside as a nest box; the remaining boxes were used fairly extensively as
sites for excretion. At 33°C mice were found in three of the four boxes, no nests were built and all boxes were used for excretion. With increasing group size nests were moved from box to box with increasing frequency and the number of boxes set aside as nest boxes was sometimes increased to two. The latter tendency appeared at group size four at 21°C and was a regular feature at group size 16 at 4°C.

Fig. 2. Number of times each male was found with the other male (solid areas) and with the females in each group (open areas). Left-hand histograms, 4°C; centre histograms, 21°C; right-hand histograms, 33°C.
At all temperatures and all group sizes, most individuals were found at least once in both compartments and all boxes, i.e. there was no sign that the pen space became divided into several territories. However, the amount of contact between individuals in the group was far less at 33 than at 4 or 21°C. Fig. 2 illustrates the number of occasions on which each male was found with the other male and with each female in the group. At 4 and 21°C the males were found together frequently (33/40 at 4°C, 27/40 at 21°C) but at 33°C they were found together only once; i.e. although the males in this group shared the same territory, they lived a Box and Cox existence with respect to the subdivisions of the shared territory. At the two lower temperatures all females were found with both males on two or more occasions; there was therefore no evidence that certain females were associated with one male only. At 33°C associations between the two males and the females were less frequent than at 4 and 21°C and several females were found with one male but not with the other.

Discussion

These results confirm the observation made on all-female groups, namely that the amount of contact between an individual and its companions is inversely related to the temperature of the environment. The increased contact at low temperatures was due in part to the accumulation of more mice, urine and woodwool in the four shelters at 4 and 21 than at 33°C. It was also due in part to the congregation of most of the animals in one or two nesting boxes. Contact with excretory products and with food handled by other mice was less in these mixed-sex groups than in all-female groups, for less food was taken to the shelters and the accumulation of faeces in these areas was reduced. Congregation of most group members in one or two nesting boxes in pens maintained at 4 and 21°C was as noticeable in mixed-sex as in all-female groups. This congregation of both one- and two-sex groups in one box and the accompanying accumulation of woodwool that occurred at 4 and 21°C probably reflects two well-known responses of rodents to exposure to low environmental temperatures, viz. (1) they build nests when the environmental temperature drops below a certain point [for rats, below 27°C (Kinder 1927)] and (2) they prefer temperatures between 31 and 36°C (Bodenheimer 1941; Stinson and Fisher 1953), and nest temperatures are usually close to this when the nests are occupied by adult mice (Barnett 1956).

The finding that mixed-sex groups have more contact with one another at 4 and 21 than at 33°C lends some weight to the suggestion that the reproductive depression observed in grouped females is due, in part at least, to female primer pheromones, and that differences in reproductive output in grouped females housed at high and low temperatures are due to differences in the amount of contact the group members have with these pheromones (Pennycuik 1972, 1973). If further experiments support this suggestion we may well have a partial explanation for the seasonal fluctuations in reproductive output observed so frequently in field populations.

Another possible mechanism by which temperatures could affect the amount of contact between individuals in a free-living situation is by regulating the size of the group sharing one territory and one nesting area. If low temperature caused otherwise antagonistic animals to form large groups as a means of conserving heat, then high temperature could cause dispersal of these animals and the formation of smaller
groups by relieving them of the necessity of seeking warmth from companions. In the experiment described above the males at 4 and 21°C showed no sign of establishing two territories. The males at 33°C shared the space available to them but they took some pains to avoid contact with one another: they were found together only once out of 40 occasions. Crowcroft (1955) found that the area dominated by one male ranged from 0·4 to 4·0 m² and Mackintosh (1970) found that male territory size ranged from 1·0 to 2·0 m². Pen size in the current experiment was only 0·25 m²; if it had been larger, the males at 33°C might well have established separate territories and halved the number of animals having regular contact with one another. Verification of this suggestion will have to await the availability of pens of suitable size.

Acknowledgments

The author would like to thank Mrs Margaret McInnes and Mrs Jillian Lovelace who collected the data presented in this paper.

References

Crowcroft, P. (1966). 'Mice all Over.' (Foulis: London.)

Manuscript received 6 March 1974