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Danilo Mainardi, Marisa Mainardi, Stefano Parmigiani, Antonio Pasquali

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Etologia. — *Relationship between Aggressiveness due to Isolation and Social Status in the House Mouse* (*). Nota (**) di DANILO MAINARDI, MARISA MAINARDI, STEFANO PARMIGIANI e ANTONIO PASQUALI, presentata dal Socio S. RANZI.

RIASSUNTO. — Nel nostro esperimento siamo partiti dall'ipotesi di lavoro che i topi mantenuti in isolamento fossero individui predominanti e che la cosiddetta iperaggressività da isolamento potesse almeno in parte essere frutto interpretativo di confronti fatti senza tenere nel dovuto conto lo stato sociale degli animali. I topi mantenuti in isolamento (che non hanno cioè sperimentato durante questo periodo esperienze di sottomissione) vengono infatti di solito confrontati con animali estratti a caso da gruppi, animali cioè che con grande probabilità si trovano in uno stato di sottomissione. Nel nostro esperimento perciò abbiamo confrontato i risultati di questo classico tipo di interazione con quelli dell'interazione tra topi isolati e topi di stato sociale alfa, ottenendo così un vero rovesciamento dei risultati per quanto riguarda l'esito dei combattimenti. Si è pensato che il maggior numero di vittorie conseguito dagli individui alfa rispetto agli isolati fosse la conseguenza del fatto che mentre gli alfa avevano conquistato il loro stato sociale attraverso una competizione, gli isolati vi erano giunti per estrazione casuale. Anche i risultati di un'altra serie di combattimenti (isolati contro « non combattenti »), l'analisi delle percentuali delle interazioni senza combattimento, e l'analisi dei rapporti tra peso corporeo e esito dei combattimenti sono in accordo con l'ipotesi che gli individui isolati sono dei predominanti.

One of the factors that apparently increase aggressiveness in the house mouse (*Mus musculus*) is isolation [1]. Furthermore, Valzelli [2] and Welch and Welch [3] have shown a positive correlation between the time spent in isolation and aggressive behavior. The size of the group also affects aggressiveness: the larger the group, the less aggressiveness there is [4, 5].

Even though the studies on aggressiveness supposedly due to isolation in the house mouse and in many other social animals are numerous [6, 7, 8], it seems to us that both in setting up the experiments and in interpreting the results, what is known about the social behavior of the species in question has not been taken into due account.

In the case of the house mouse, we deem it essential to take into consideration the fact that it is a species with group territories, in which the males organize themselves into a usually linear hierarchy [9]. That is, whenever a group of male adult mice is stabled together for long enough, a sort of behavioral polymorphism develops, with each individual characterized by its social status. To simplify, as would seem useful at least in a preliminary approach, in every stable group of male mice it is possible to distinguish

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a dominant individual and a group of submissive individuals. The status of social dominance confers on its holder well-defined and differentiated characteristics as regards both sexual behavior and the defense of territory [9]. Keeping this in mind, if we consider how the experiments from which the hyperaggressivity of the isolated mouse was deduced were set up, substantial perplexities arise. Usually the animals kept in isolation are compared (regarding both behavior and hormone levels) with individuals taken at random from social groups, and hence likely to be submissive. This likelihood increases with the size of the group from which said individual is taken. If we define submissive animals as those who have experienced submission, and dominant individuals as those who have not, obviously referring in both cases to their most recent social experiences, it is clear that, in this possibly oversimplified dichotomy, the isolated individuals fall into the category of dominant individuals.

It was this experimental hypothesis that led to the present study, consisting in the comparison of the aggressive behavior of isolated and dominant mice. As a control, other isolated mice were compared with mice drawn at random from groups.

Altogether, 356 random-bred male albino mice (Swiss strain) from the Morini farm of San Polo d'Enza (Reggio Emilia) were used in this study. They were stabled as described below, from the age of 25 days. One hundred and twenty mice were kept in isolation for 6 weeks in opaque plexiglass boxes ($13 \times 27 \times 12$ (h) cm). The remaining 236 males were kept in groups of 10–15 individuals in $30 \times 40 \times 17$ (h) cm opaque plexiglass boxes.

For the control tests, individuals that had been isolated for 6 weeks were tested with individuals taken at random from the groups. For the tests with an isolated and a dominant mouse, the procedure was as follows: five weeks after the beginning of stabling, pairs were formed by random extraction from two different groups. Each pair was placed in a box and observed while social dominance was established. The two males were left together, and, at the end of the sixth week the dominant one was tested with an isolated mouse. The dominant males were chosen from such tiny hierarchies (that is, one of only two individuals: one dominant and one submissive) to give the least possible weight to selection through competition for social status, since these dominant animals were to be compared with the isolated mice, which are presumed to be dominant not by merit of competition, but by chance.

In a certain number of cases, the two males extracted from the social groups did not fight at all, rendering it impossible to detect their hierarchical order, if there was one. These mice were tentatively classified as " noncombatant " and were used in a third series of tests with the isolated individuals.

Before the behavioral tests, the mice were weighed, and those under 20 g discarded. The behavioral test consisted in placing each pair in a fighting box. A fighting box is a $20 \times 25 \times 12$ (h) cm parallelepiped in opaque plexiglass (the bottom covered with sawdust) with a dividing screen on the diagonal, separating the fighting area into two equal parts. A mouse was placed in each half, the dividing screen was removed 24 h later, and the behavior of the two animals was observed and recorded for an hour.

The three categories of fights were:

a) isolated males against males drawn at random from groups;
b) isolated males against alpha males from a small hierarchy;
c) isolated males against " noncombatant " males.

Results of Fights.

Out of 40 category-*a* fights, the isolated male came out dominant 21 times, the male from the group 12 times, and in seven cases there were no signs of fighting.

The situation is quite different in category *b*: in fact the isolated male dominated in 11 out of 33 encounters as opposed to the 22 times in which the alpha male did. There was fighting in all cases.

Restricting ourselves to the cases in which fighting occurred, a comparison of these two categories shows that the cases in which the isolated mouse acquires dominance go from 63.6% in category *a* to 33.3% in category *b*. The difference is statistically significant ($\chi^6 = 4.91$; $P < 5\%$).

This is probably the most important result of our experiment. In fact, whereas the outcome of the fights in category *a* seems to substantiate the widely held thesis of an increase in aggressiveness in isolated mice, the outcome of the fights in category *b* reverses the results: when compared with males that showed dominance in competition with other males, the isolated males win fewer fights. This is not surprising as, even though our experimental hypothesis considers isolated mice to be dominant, they are " dominant by chance ", whereas their competitors in category *b* conquered their alpha position by fighting and defeating another male, that is not by chance.

Out of 19 encounters in category *c*, the isolated mouse established dominance 11 times, a " noncombatant " male did once, and in 7 cases there was no fighting.

A comparison of the percentage of cases in which there was no fighting in the three categories (17.5% in *a*, 0% in *b* and 36.8% in *c*) shed further light on the results. Neither the difference between the 17.5% of *a* and the 0% of *b* ($P = 0.01$) nor that between the 0% of *b* and the 36.8% of *c* ($P = 0.0004$) can be ascribed to chance, whereas the difference between categories *a* and *c* is not significant. In our opinion the obvious explanation of the difference between *b* on the one hand and *a* and *c* on the other regarding the cases of noncombativeness lies in the fact that in category *b* the encounter is always between two dominant individuals, whereas this is not the case in the other two categories.

Relationship Between Weight of the Mice and Outcome of the Fights.

Further information can be obtained by considering the relationship between the weight of the animals and the outcome of the fights. In the first place, in experimental situations *a* and *b*, the individuals that dominated were on the average significantly heavier than those that submitted. The mean weight of the former was 33 g (s.e. = 0.44) and that of the latter 29 g (s.e. = 0.56) ($t = 5.63$; $P < 1\%$).

Instead, when one considers the animals that did not fight in situations *a* and *c*, one finds no difference in weight: in category *a* the individuals

that did not fight weighed an average of 26.9 g (s.e. = 0.71) and in category *c* 28.7 g (s.e. = 0.08) ($t = 0.41$; $P > 5\%$). A lack of significant weight differences is also found when comparing the " noncombatants " with their group of origin. In fact the isolated mice had a mean weight of 28.1 g (s.e. = 0.97) and those in groups 27.5 (s.e. = 0.91) ($t = 0.47$; $P > 5\%$).

A comparison between the weights of the isolated mice and the others in situations *a* and *b* also gives interesting results. In *a*, there is no significant difference between the mean weights: the isolated mice have an average-weight of 30.5 g (s.e. = 1.03) and those from groups 29.7 g (s.e. = 0.77) ($t = 0.08$; $P > 5\%$). Instead, in situation *b*, the mean weight of the mice taken from groups is significantly greater than that of the isolated mice. The former weight 32.8 g (s.e. = 0.57) on the average, and the latter 30.03 g (s.e. = 0.78) ($t = 2.92$; $P < 1\%$). It should be kept in mind that, in category *a*, the mice from groups were chosen at random, whereas those in *b* had won in a fight between two individuals taken at random from groups. Thus the greater weight of these animals as opposed to the isolated ones in situation *b* can help us to understand the results in this situation. It will be recalled that, in *b*, the isolated males won fewer battles than their competitors. Our working hypothesis is that the isolated males are " dominant by chance " simply because they have had no recent experiences of submission. It was therefore to be expected that, pitted against males that had won their dominance in aggressive competitition, they would be unequally matched. In fact, the males that had already succeeded in winning a fight (the partners of the isolated males in *b*) weighed significantly more than the isolated ones, since greater weight is one of the factors facilitating victory (a fact that can also be seen from our overall results), and this greater weight is, in turn, likely to have been one of the factors determining the large number of defeats by the isolated mice.

Another point of interest lies in the cases in which, contrary to the general trend, the lighter individuals won against their heavier opponents. In category *a*, of the 21 isolated males that won, 11 were lighter than their opponents (52.38%), whereas only 2 out of the 12 (16.66%) winning males taken at random from groups were lighter than their opponents. Fisher's exact probability test shows the probability of such a difference occurring by chance to be 3 in a thousand. This difference is maintained in the same direction in situation *b* where 6 out of the 11 isolated mice that won (54.5%) were lighter than their opponents, whereas only 1 out of 22 (4.5%) of the alpha mice from small hierarchies defeated a heavier partner (isolated mouse). There are 2 probabilities in a thousand that this difference was due to chance. Regarding the interpretation of these results, it is likely that in a social situation light-weight individuals have a slight chance of becoming dominant, whereas weight is obviously of no importance when dominance is due to isolation. Once dominant social status has been obtained, it must be concluded that weight no longer has much influence on the outcome of combat.

Conclusions

The starting point of our experiment was the working hypothesis that mice kept in isolation are dominant individuals and that so-called hyperaggressiveness due to isolation is, at least partly, a mistaken interpretation of comparisons made without taking the social status of the animal into proper account. In fact, mice kept in isolation (who therefore have not had submissive experiences during this period) are usually compared with animals taken at random from groups, and thus likely to have a submissive status. Therefore in our experiment we compared the results of this classical type of interaction with that of the interaction between isolated mice and mice with alpha social status, obtaining a reversal of results regarding the outcome of fights.

It seems likely that the greater number of victories won by alpha individuals in comparison with the isolated males was due to the fact that, whereas the alphas had won their dominant status through competition, the isolated males had been granted it by chance. Both the analysis of another series of fights (isolated males against " noncombatants ") and the analysis of the percentages of encounters without fighting in the various experimental situations support the hypothesis that isolated individuals are dominant individuals. The same is true of the analysis of the relationship between the weight of the mice and the outcome of the fights. Further confirmation of the hypothesis that isolated mice are in a condition homologous to that of social dominance is provided by the research of Jones and Nowell [10], who demonstrated that mice kept in isolation have the pheromone characteristic of dominant males, that of Crawley, Schleidt and Contrera [11], who found that males stabled only with females fight as much as isolated males, whereas males stabled in groups of males or males and females fight much less, and, lastly, that of Hucklebridge, Reid, Benton and Brain [12], who showed that the levels of adrenal catecholamine in isolated mice resemble those of dominant mice more than those of submissive ones.

In conclusion, it should be remembered that the results on so-called hyperaggressiveness due to isolation have been used to support Lorenz's much discussed theory of appetence for aggression (see [7], [8], [13], [14], [15], among others). The results of the present experiment and our interpretation of it do not support this theory.

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