

EFFECTS OF CASTRATION ON POSTNATAL DIFFERENTIATION IN THE JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*)

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In the Japanese quail, adult males and females are dimorphic for behaviour. When tested with a receptive female, males show the following behaviour sequence: neck grab, mount, cloacal contact movement (CCM) and strutting, whereas females never perform male sexual behaviour and when isolated, they never crow as males do. Peripheral structures are also sexually dimorphic. The cloacal gland, an androgen dependent glandular structure behind the cloaca is well developed in males, but remains rudimentary in females. Furthermore, the development of the sternotracheal muscles (syringeal muscles) depends on androgens and the muscles are lighter in females than in males. These sternotracheal muscles play a role in frequency modulation of crowing (1). These behavioural and morphological differences between sexes are not only controlled by the different adult endocrine status, but are based on more stable differences. Indeed, testosterone (T) which restores sexual behaviour in castrated males, fails to elicit copulation in ovariectomized females and T-treated females have smaller cloacal glands and lighter sternotracheal muscles than males treated with the same amount of hormone (2, 3).

In the Japanese quail, sexual differentiation appears as a demasculinization of females. It is believed that this demasculinization of behaviour and morphology results from exposure of the female quail to estrogens released by the embryonic ovary before day 12 of incubation. Injections of oestradiol benzoate (EB) in the egg demasculinizes male embryos only if the steroid treatment is given before day 12 of incubation (4-6). Injections of the anti-oestrogen CI-628 on day 9 of incubation in female eggs prevent their demasculinization (7). These data suggest that the process of sexual differentiation is completed before hatching in quail. More recently, Hutchison (8) demonstrated that if female quail are ovariectomized on the day of hatching and injected as adults with testosterone propionate (TP), a significant proportion of females show male sexual behaviour. We also showed recently that if females are ovariectomized as late as one week after hatching and treated with T when adult, almost 50% (6 out of 13) show cloacal contact movements and 10 out of 13 perform neck grabs. Furthermore, these neonatally gonadectomized females grow cloacal glands of the same size as those of neonatally castrated males (9). These experiments show that the demasculinization of female quail is not completed at the time of hatching.

Materials and methods: To analyze the progress of female demasculinization during the postnatal life, we castrated males and females at 1 day or at 1, 2, 4 and 6 weeks after hatching. The birds came from 3 different batches and males and females from each batch were distributed in a random order between the 5 experimental groups. Birds were raised in mixed groups under long days (16 L: 8 D, light on at 06.00 h). At the age of 2 months, the quails were isolated in individual cages and received 60 mm of subcutaneous silastic implants of T (Dow corning silastic tubing nbr. 602-265, 1.57 mm i.d., 2.41 mm o.d.; T: Sigma no. T-1500). We showed previously that this treatment induces copulation in 100% of castrated males (3). Three weeks later, the birds were tested with a receptive female twice in a test arena (30 by 80 by 60 cm height) whose floor was covered with sand and once in their home-cages during 5 min each time. On the day of sacrifice (4 weeks after implantation), the cloacal gland area of each bird was measured and the 2 sternotracheal-muscles were excised and weighed to the nearest milligram.

Results and discussion: Cumulative results for all the behavioural tests are shown in figure 1. Females castrated at or before the age of 2 weeks showed a higher incidence of male type sexual behaviour than those castrated later in life (Fisher's exact probability test, two-tailed: $P < 0.02$ for mount attempt, mount and CCM) but this difference was not observed in males ($P > 0.60$ for each behaviour pattern). As a consequence, the sexual behaviour of males and females was significantly different at 4 and 6 weeks of age (see figure 1) but not before.

Cloacal glands were significantly smaller in females than in males when birds had been gonadectomized at one week of age or later and the magnitude of this sex difference tended to increase when gonadectomy was performed later (see figure 2). This was due to the fact that the cloacal glands of males did not change with the age of castration ($F = 0.799$; $df = 4, 22$; $P = 0.540$; exact probabilities from a Radio-Schack TRS-80 analysis of variance program) whilst the glands of females

tended to be smaller if ovariectomy was performed later in life although the effect was not significant ($F = 2.077$, $df = 4,33$, $P = 0.105$).

The differentiation of the sternotracheal muscles seems to occur even later in life and is irreversibly achieved only 2 weeks after hatching. The sexual differences result from a decrease in the muscle size in females when ovariectomy is performed at or later than 2 weeks (variance analysis of muscle weight at different ages: females, $F = 3.596$, $df = 4,32$, $P = 0.016$; males, $F = 0.689$, $df = 4,22$, $P = 0.623$).

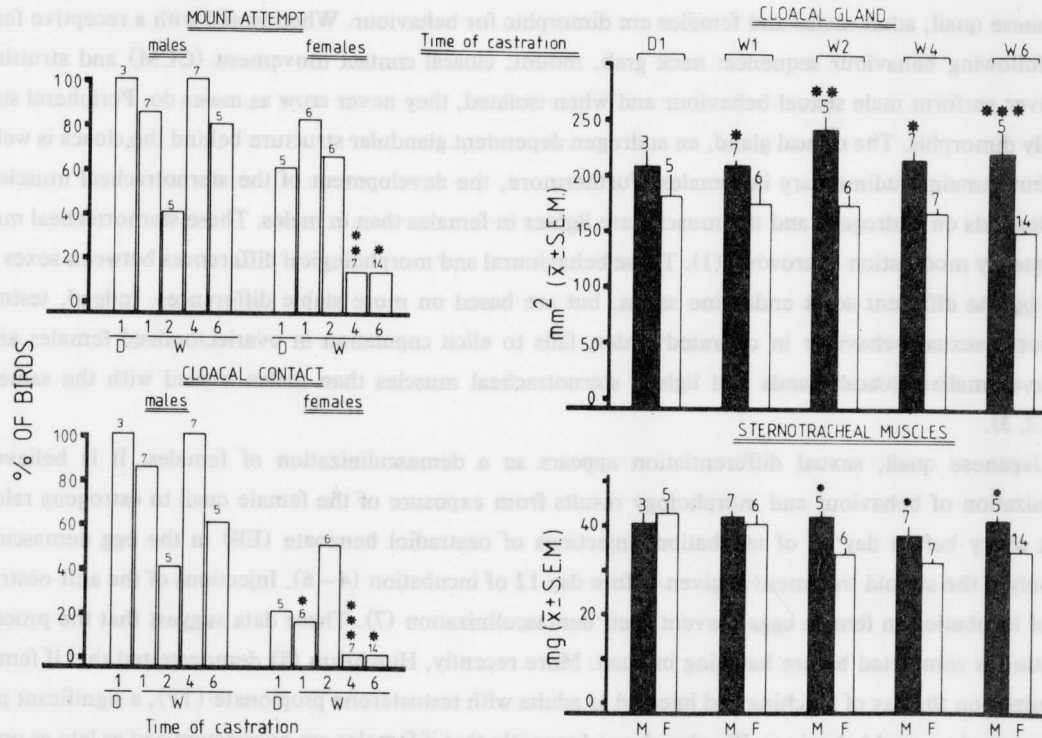


Figure 1 (left): Percentage of males and females who attempted to mount and who performed cloacal contact movements. Numbers above the columns indicate the number of animals in each group. In comparison with males in the corresponding group $P < *0.05$; $**0.01$; $***0.001$ (Fisher's exact probability test, two tailed). D = day; W = week. Figure 2 (right): Cloacal gland area and weight of the sternotracheal muscles in males and female quail gonadectomized at different ages after hatching. Numbers above columns indicate the number of birds in each group. In comparison with females of the same group $P < *0.05$; $**0.01$; $***0.001$ (Student's *t* test, two tailed). D = day; W = week; M = male; F = female.

This experiment shows that during postnatal development, there is no masculinization by testicular hormones of sexual behaviour and morphology in males because deprivation of testosterone by castration performed as early as day one of life does not alter subsequent responsiveness to exogenous testosterone. Indeed, there were no differences in copulatory behaviour, cloacal gland area and weight of sternotracheal muscles between males castrated as soon as 1 day and as late as 6 weeks after hatching. Sexual differentiation in quail thus appears as a demasculinization of females by ovarian secretions. This demasculinization of females is a continuous process which is completed at different stages of development for the different androgen dependent structures.

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