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# GENETIC TRENDS IN DOE AND KIT BEHAVIOUR AND PERFORMANCES ASSESSED WITH COMPARISON OF OLD AND MODERN-TYPE LINES IN A CROSSFOSTERING DESIGN

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## ABSTRACT

The study focused on the estimation of genetic trends for behavior and performances of does and kits during lactation in parallel to 22 generations of selective breeding for litter size and kit growth. The direct effects from the kit (kit line) were separated from the maternal effects of the doe (doe line) with use of a crossfostering design between the old-type (L0) and the modern-type (L22) lines. Does were studied over the two first parities. They raised rabbits from a single kit line. None kit was raised by its biological mother. At d21, L22 females produced more milk than L0 females (250 g vs 206 g,  $p=0.0003$ ) which resulted in a higher weight of the kits at d21 (378 g vs 340 g,  $p<0.0001$ ). L22 does had better maternal abilities than L0 does as referred to nest quality ( $p=0.06$ ), fur puckling ( $p<0.0001$ ), milk production ( $p=0.0003$ ) and willingness to nurse ( $p=0.007$ ). At most dates, more L22 kits were observed out of the nest than L0 kits, whether they were raised by L0 or L22 does. L22 kits exited the nest earlier during lactation and were bolder in an emergence test than L0 kits. Trends in doe behaviour were positive and favorable to litter performance. In advanced lactation, genetics of the kit influenced kit behaviour more than genetics of the nursing dam.

**Key words:** maternal behavior, kit, doe, breeding, genetic trends, boldness

## INTRODUCTION

Perinatal mortality of kits is a major economic and ethical issue that cannot be reduced by selection due to the low heritability of mortality traits (Garreau et al., 2015). Selection for direct and maternal effects of weaning weight may be an alternative to reduce kit losses and improve their welfare. Garreau et al. (2015) demonstrated that this breeding goal led to select individuals from litters with low mortality and thus contributed to improve kit survival. We assume that selection for direct effects of kit growth may increase their nutrient needs and modify their behaviour related to milk intake. In addition, selection for maternal effects may result in improvement of maternal skills. The objective was to estimate changes in doe and kit behaviour and performances with comparison of an old-type line (L0) to a modern-type line (L22), and to quantify the contribution of direct effects and maternal effects on kit performance and behaviour.

## MATERIALS AND METHODS

### Animals and experimental design

Rabbits were produced and raised in a Hypharm commercial farm (GRIMAUD). Harsh housing condition was induced by the protocol: with early manipulation of kits with adoption and mixing at d1, without sacrifice of weak kits, and nest maintained at advanced stage of lactation. The last procedure

is likely detrimental to the sanitary status of cages. Two lines are studied: the L22 modern-type line, selected for 22 generations for prolificacy, and direct and maternal effects for weaning weight (Garreau et al., 2005) and the L0 old-type line produced from progeny of frozen embryos (Joly et al., 1998) of the ancestor population of the selected line. Data collection was performed from a single batch at each parity. Therefore, behaviour and performance were recorded on two cohorts of females 6 weeks apart: on 52 L0 and 59 L22 females in parity 1 and among them, 28 L0 and 29 L22 females from the first batch including parity 2 females were studied. A crossfostering design enabled to disentangle direct effects of the kit from maternal effects of the dam on performances and behaviour. It was defined so that none kit was raised by its biological mother. Each litter included kits from a single line born from 4 to 9 litters of origin. Litters given to does were uniform in kits weights and included 7 kits in 1<sup>st</sup> parity and 8 kits in 2<sup>nd</sup> parity for half of the does, and respectively 8 and 9 kits for the other half. L0 females raised either a litter of L0 kits or a litter of L22 kits and similarly for L22 females. Nursing was controlled with manual opening of the nest from d1 to d11 of lactation. Afterwards, the hatch was kept open: the nest and doe area were accessible permanently to does and kits except at d21 when milk production was measured by weighing of the doe before and after nursing (hatch closed the day before and opened manually for nursing). Kits were weighed and earmarked the day of birth, and weighed again on d21 and d35 (weaning). Kit survival was recorded daily until weaning.

### **Behavioral measurements**

Nest quality was assessed the day before (d0) and the day of farrowing (d1) with presence of a clearly visible nest made with fur, or not. Fur plucking by the doe was observed as complete or not (Burri, 2014). Doe willingness to nurse was assessed from doe behaviour before and after nursing on d5 and d8 of lactation, through doe position in the cage and activity before the opening of the nest, as well as whether it entered the nest on its own or not. Doe's reaction to litter removal for weighing was analysed on d6 and d28 of lactation, as a positive response (scratch the door, stand on its hind legs, step forward) or a non-positive response (retreat when human approach, absence of reaction). From d12 to d29 of lactation, the number of kits present in the doe's compartment was counted at 10 a.m. Behavioural notations were undertaken on the 2 first parities. A device to measure kit boldness in a novel environment, referred to as an emergence test, was developed from Zueca et al (2012). It included an habituation box followed by a first arena free of access and next a second arena where the kits had to path through an opening similar to the hatch. The test was performed on 2 kits randomly chosen in the litter but one of low weight and the other of high weight compared to the litter average weight, at d22 and d25 of lactation in 1<sup>st</sup> parity. Tested kits were first individually placed for 2 minutes in the dark opaque habituation box. The test lasted 3 min. At the opening of the hatch, the latency to go to the first arena, the time spent in each arena and the number of times a kit put its head or front leg only through the aperture (partial intrusion) of each arena were recorded.

### **Statistical Analysis**

Performance and kits behaviour data were analysed with a linear model taking into account the doe line, the kit line, the doe parity and for kit emergence the day of observation. In addition, the number of born alive and the number of kits adopted were included in all analyses except that for the mortality rate. Kit weight and milk production at d21 were adjusted for litter size at d21. Kit weight and litter weight gain at weaning were adjusted for the number of weaned kits. Logistic regressions were applied for the analysis maternal behaviour. The model for nest quality accounted for the interaction between the doe line and parity and day of observation. For the other traits, the fixed effects included the doe line, the kit line (except for fur plucking), parity (except for willingness to nurse) and the day of observation. The doe random effect was included in all analyses. Results are least square means estimated with R software (Team R Core, 2016).

## RESULTS AND DISCUSSION

L22 females produced more milk than L0 females (250 g vs 206 g,  $p=0.0003$ ) which resulted in a higher growth of the kits they raised as regards to kit weight at d21 (378 g vs 340 g,  $p<0.0001$ ). The litter mortality rate in lactation was lower in L22 females (13 vs 22%  $p=0.03$ ) (Table 1). The genetic trends we observed with this line comparison are in the same direction but lower than the estimates from Garreau et al. (2015), certainly due to the protocol causing a harsh environment. Interestingly, direct effects on kit growth were visible after d21 only, with L22 kits growing faster than L0 kits in each doe line. Also, we confirmed that selection for kit growth indirectly reduced kit mortality.

**Table 1:** Least square mean performance of doe and kit from the old line (L0) and modern-type line (L22)

| Doe line                                   | Parity          | L0 n = 52 (P1) n = 28 (P2)  |                               | L22 n = 59 (P1) n = 29 (P2)   |                             |
|--|-----------------|-----------------------------|-------------------------------|-------------------------------|-----------------------------|
|  |                 | L0<br>n = 179; n = 120      | L22<br>n = 210; n = 118       | L0<br>n = 195; n = 120        | L22<br>n = 247; n = 127     |
|  |                 | Mean $\pm$ s.e.             | Mean $\pm$ s.e.               | Mean $\pm$ s.e.               | Mean $\pm$ s.e.             |
| Kit weight at d21 (g) <sup>δ2</sup>        | 1 <sup>aΨ</sup> | 327 $\pm$ 8 <sup>a</sup>    | 325 $\pm$ 8 <sup>a</sup>      | 365 $\pm$ 7 <sup>b</sup>      | 363 $\pm$ 6 <sup>b</sup>    |
|  | 2 <sup>b</sup>  | 354 $\pm$ 8 <sup>a</sup>    | 353 $\pm$ 9 <sup>a</sup>      | 393 $\pm$ 8 <sup>b</sup>      | 391 $\pm$ 9 <sup>b</sup>    |
| Kit weight at weaning (g) <sup>1</sup>     | 1 <sup>a</sup>  | 598 $\pm$ 13 <sup>a</sup>   | 675 $\pm$ 13 <sup>b</sup>     | 650 $\pm$ 13 <sup>b</sup>     | 727 $\pm$ 13 <sup>c</sup>   |
|  | 2 <sup>b</sup>  | 671 $\pm$ 15 <sup>a</sup>   | 747 $\pm$ 15 <sup>b</sup>     | 723 $\pm$ 15 <sup>b</sup>     | 799 $\pm$ 15 <sup>c</sup>   |
| Litter weight gain d1-d32 (g) <sup>1</sup> | 1 <sup>a</sup>  | 3480 $\pm$ 142 <sup>a</sup> | 3880 $\pm$ 154 <sup>a,c</sup> | 3934 $\pm$ 133 <sup>b,c</sup> | 4335 $\pm$ 144 <sup>b</sup> |
|  | 2 <sup>b</sup>  | 4031 $\pm$ 154 <sup>a</sup> | 4431 $\pm$ 145 <sup>a,c</sup> | 4486 $\pm$ 158 <sup>b,c</sup> | 4886 $\pm$ 148 <sup>b</sup> |
| Milk production d21 (g) <sup>2</sup>       | 1               | 216 $\pm$ 11 <sup>a,c</sup> | 192 $\pm$ 11 <sup>a</sup>     | 260 $\pm$ 11 <sup>b</sup>     | 236 $\pm$ 11 <sup>b,c</sup> |
|  | 2               | 220 $\pm$ 12 <sup>a,c</sup> | 196 $\pm$ 12 <sup>a</sup>     | 265 $\pm$ 12 <sup>b</sup>     | 240 $\pm$ 13 <sup>b,c</sup> |
| Litter mortality rate (%)                  | 1               | 24 $\pm$ 4 <sup>a</sup>     | 25 $\pm$ 4 <sup>a</sup>       | 15 $\pm$ 4 <sup>b</sup>       | 16 $\pm$ 4 <sup>b</sup>     |
|  | 2               | 19 $\pm$ 4 <sup>a</sup>     | 21 $\pm$ 4 <sup>a</sup>       | 10 $\pm$ 4 <sup>b</sup>       | 12 $\pm$ 4 <sup>b</sup>     |

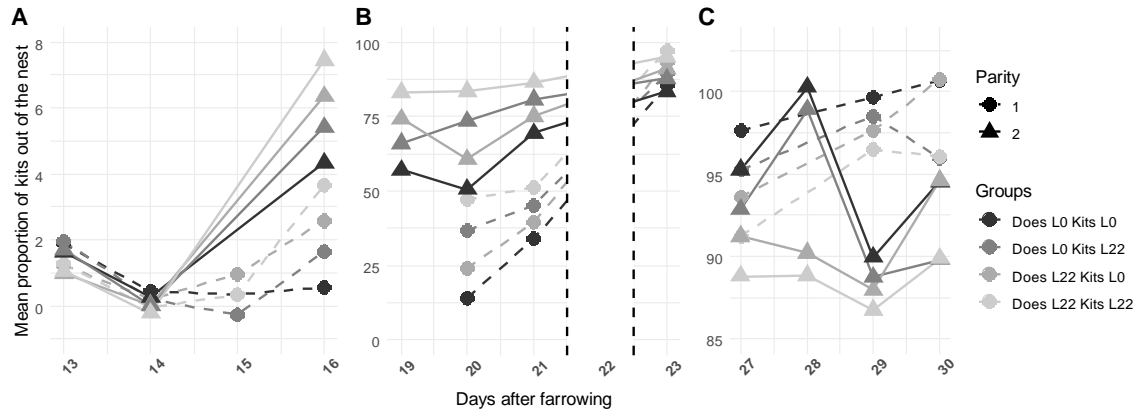
<sup>δ</sup> correction for : <sup>1</sup> litter size, number born alive and number of weaned kits, <sup>2</sup> litter size, number born alive and litter size at d21

<sup>Ψ</sup> different letters (a,b,c) indicate a significant difference ( $p < 0.04$ )

Nest quality improves from d0 to d1 ( $p<0.01$ ). In first-parity on d0, the probability that L0 females have a better nest quality is 19% compared to 58% for L22 does ( $p=0.008$ ). On d1, the probability increases to 46% for L0 does and 84% for L22 does ( $p=0.008$ ). In second parity, there are significant differences between d0 and d1 (21% in d0, 64% in d1,  $p=0.0009$ ) but no between female-line. The fur appearance of the female is in line with the nest quality results, with a fur plucking more important in L22 females than in L0 females at both dates and both parities ( $p < 0.0001$ ). In first parity, the probability that L0 females make fur plucking is 36% on both d0 and d1 and that of L22 does is 58% and 56%. In second parity, at d0-d1, the probability of make fur plucking is 38-36% in L0 females and 61-63% in L22 females. The L22 does start building a nest with use of their fur earlier than L0 does. In first-parity on d5, the probability to be close to the hatch before nursing is higher 44% in L0 does and 70% in L22 does ( $p=0.04$ ). The probability on d8 increases to 80% for L0 and 92% for L22 does. In first-parity on d5, the probability that females enter the nest alone when the hatch is opened is similar in L0 (55%) and L22 (57%). This probability increases at d8 to 89% for L0 and 92% for L22. The increased willingness to nurse within parity1 indicates accustomization to controlled procedure and/or increased need for nursing. In second parity, on any date, all L0 and L22 does enter the nest alone and are close to the hatch before nursing, which emphasize the effect of previous maternal experience. The tendency for L22 does to be more attached to their litter than L0 as measured by their reaction to handling of the kits ( $p = 0.11$ ) is in line with previous results.

First kit exit from the nest takes place around d13. At d20, the proportion of kits observed out of the nest is 31% for L0 kits and 68% for L22 kits independent of the doe line ( $p = 0.006$ ). This proportion increases to 52% for L0 kits and remains stable in L22 kits on d21, and to 96% and 98% for L0 kits and 99% and 93% for L22 kits on d26 and d30 respectively. This effect of kit line is significant ( $p = 0.08$  at d21 and  $p < 0.04$  on other days). On d21, d29 and d30, the influence of parity was significant ( $p < 0.009$ ): there are more kits observed out of the nest on d21 and less on d29 and d30 in second parity.

At d23 and d28, it is the effect of the doe line that impacts the nest exit ( $p = 0.04$ ). The proportion of kits emerging from the nest is then 87% and 100% respectively for kits raised by L0 does and 94% and 89% for kits raised by L22 does (Figure 1). In the emergence test, the time needed to exit the habituation box (on average  $47 \pm 7$ s) and to exit arena 1 ( $140 \pm 7$ s) is similar for L0 and L22 kits. The time spent in the habituation box (78s) and in arena 1 (77s) is similar between L0 and L22 kits. However, L22 kits spend more time in arena 2 on d25 than on d22 (13s vs 43s,  $p = 0.01$ ), as well as kits raised by L0 does (11s vs 36s,  $p = 0.03$ ). L22 kits make fewer partial intrusions into arena 1 at d25 than at d22 (1.4 vs 0.4,  $p = 0.006$ ), and spend less time in the habituation box (91s vs 57s,  $p = 0.14$ ).



**Figure : 1** Trends in kit exit of the nest, on d13 to d16 (A), d19 to d23 (B), d27 to d30 (C)

This last association shows that L22 kits are less hesitant to enter in arena 1 at d25. Kits raised by L22 females make more partial intrusions into arena 2 at d25 than at d22 (2.2 vs 4.5  $p = 0.03$ ), which indicates that they are less fearful the second time (Zueca et al., 2012). L22 kits leave the nest earlier than L0 kits and, in agreement, they are more inclined to the visit of unknown areas in the emergence test. Large effects of the kit line on kit behaviour are detected. The overall results show more marked maternal effects in the start of lactation. With effects (in favor of L22) on growth (greater weight of kits on d21) and on females behaviour, with better nest preparations and a stronger motivation to nursing from the first parity. In addition, the direct effects are more marked at the end of the lactation period with an influence on the weight gains of the litter, an earlier nest exit and a more marked curiosity. All by having progressed on the number of births alive (8 vs 10  $p = 0.02$ ) and the litter mortality.

## CONCLUSIONS

Selection for kit growth and litter size was efficient with a greater weight gain, a better milk production and a lower mortality rate during lactation for kits raised by L22 females. It was accompanied by improvement of maternal abilities and modern-type females being more responsive to their kits. L22 kits leave the nest earlier and they are more curious than L0 kits. Kit growth and behaviour in late lactation is influenced mainly by direct effects.

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