# Correlated response to selection for environmental variability of litter size in rabbits' resilience

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Short title: Correlated response in rabbits' resilience

## Abstract

Resilience is the ability of an animal to return soon to its initial productivity after facing diverse environmental challenges. This trait is directly related to animal welfare and it plays a key role in fluctuations of livestock productivity. A divergent selection experiment for environmental variance of litter size has been performed successfully in rabbits over ten generations. The objective of this study was to analyse resilience indicators of stress and disease in the divergent lines of this experiment. The high line showed a higher perinatal mortality than the low line (+4.1%). After correcting by litter size, the difference was +3.2%. Involuntary

culling rate was also higher in the high than in the low line (+12.4%). Before vaccination against viral haemorrhagic disease or myxomatosis, concentration of lymphocytes, C-reactive protein (CRP), complement C3, serum bilirubin, triglycerides and cholesterol were higher in the high line than in the low line (difference between lines +4.5%, +5.6 µg/mL, +4.6 mg/mL, +7.9 mmol/L, +0.3 mmol/L and +0.4 mmol/L). Immunological and biochemical responses to the two vaccines were similar. After vaccination, the percentage of lymphocytes and CRP concentration were higher in the low line than in the high one (difference between lines +4.0% and +13.1 µg/mL). The low line also showed a higher increment in bilirubin and triglycerides than the high line (+14.2 vs +8.7 mmol/L for bilirubin and +0.11 vs +0.01 mmol/L for triglycerides); these results would agree with the protective role of bilirubin and triglycerides against the larger inflammatory response found in this line. In relation to stress, the high line had higher basal concentration of cortisol than the low line (+0.2 ng/mL); the difference between lines increased more than three-fold after the injection of ACTH, the increase being greater in the high line (+0.9 ng/mL) than in the low line (+0.4 ng/mL). Selection for divergent environmental variability of litter size leads to dams with different culling rate for reproductive causes and different kits' neonatal survival. These associations suggest that the observed fitness differences are related to differences in the inflammatory response and the corticotrope response to stress, which are two important components of physiological adaptation to environmental aggressions.

**Keywords:** C-reactive protein, cortisol, environmental variability of litter size, resilience, rabbits

## Implications

Livestock industry is increasingly interested in having dams more resilient and robust. Litter size variance within female has been proposed as a selection criterion to improve female's resilience. This study evaluates the correlated response in environmental sensitivity of two lines divergently selected for litter size variability. The high line showed higher perinatal mortality and larger involuntary culling rate than the low line, which would agree with a different susceptibility to stress and disease between lines. Therefore, selection to reduce litter size variability could lead to more resilient animals.

## Introduction

Genetics selection has considerably increased the productivity in the livestock species, but it is questioned concerning its negative effect on welfare (Rauw *et al.,* 1998). In this scenario, livestock industry is increasingly interested in having animals more adapted to environmental perturbations. Robustness and resilience are individual characteristics favouring welfare. Robustness has been defined as the ability of the animal to express its production potential in a wide range of environments without compromising its health and reproduction (Knap *et al.*, 2005). Resilience is the capacity of the animal to rapidly return to the physiological, behavioural, cognitive, health, affective and production states that pertained before exposure to a disturbance (Golditz and Hine *et al.,* 2016). Both robustness and resilience are genetically related to environmental sensitivity (Mirkena *et al.,* 2011 for robustness; Mulder and Rashidi, 2017 for resilience). In the case of litter size, a trait directly related to fitness, environmental variability of litter size would be related to both the ability to maintain a relatively undepressed

production level during infection, and the ability to face the invading pathogen's and subsequent development of infection (i.e. tolerance and resistance). Mulder and Rashidi (2017) found that selection on resilience increases disease resistance and tolerance to infections; therefore, improving of resilience may lead to improving robustness.

A divergent experiment for environmental variability of litter size has been performed successfully in rabbits during ten generations, the line selected for increased environmental variability of litter size showing a greater variability (4.4 kits$^2$) than the low line (2.7 kits$^2$) (see Blasco *et al.,* 2017). Furthermore, selection for environmental variability of litter size displayed a negative correlated response with litter size (7.8 kits in the high line vs 8.5 kits in the low line), due to a lower early embryo development (García *et al.,* 2016) and a lesser success in embryo implantation rate (10.4 implanted embryos in the high line vs 11.7 implanted embryos in the low line, Argente *et al.,* 2017). We hypothesize that there are two possible mechanisms acting in variability of litter size. The first one at organ level, related to uterine aptitudes, and the second one at animal level, related to the ability of the female to cope during its gestation with environmental challenges, including chronic stressors and infectious diseases. In relation to the first mechanism, there is evidence that maternal stress during peri-implantation period increases embryonic losses (Burkuš *et al.,* 2015), limiting the uterus' capacity to gestate the maximum potential number of embryos at term; therefore, variability of litter size can be related to the uterine capacity of the female. In this sense, Ibáñez-Escriche *et al*. (2008) reported a negative correlation between uterine capacity (a trait highly correlated with litter size, Argente *et al.,* 2000) and

its residual variability. In relation to the second mechanism, we hypothesize that dams with a greater ability to cope with environmental changes would show less sensitivity to stress and diseases, and in consequence less litter size variability. If so, environmental variability of litter size would be related to doe's resilience.

It is well established that the hypothalamic–pituitary–adrenocortical (HPA) axis is activated in response to environmental stimuli, releasing glucocorticoids to bloodstream, and having a key role in coping with environmental challenges (Janssens *et al.,* 1995). Serum cortisol concentration has been used traditionally as a biomarker of stress in domestic animals (Cabezas *et al.,* 2007). Besides, it is known that chronic stress leads to dysregulation of the immune system (Webster *et al.,* 2002), increasing predisposition to disease (Glaser and Kiecolt-Glaser, 2005). Concerning diseases, acute phase proteins are adequate biomarkers of disease sensitivity, since these plasma proteins change quickly their concentration in response to an inflammatory or infectious process (Eckersall, 2000). The concentration of lymphocytes and neutrophils in blood may also be used as markers of inflammatory response (Madjid and Fatemi, 2013; Yaşar *et al.,* 2016).

The objective of this paper is to analyse resilience indicators of stress and disease in the lines of the divergent experiment for environmental variance of litter size mentioned before, in order to find whether selection produced a correlated response in resilience.

## Material and Methods

126

127 *Animals*

128 Rabbits used in this study came from a maternal synthetic line created from
129 commercial crossbred does. Rabbits were bred at the farm of the Universidad
130 Miguel Hernández of Elche. Reproduction was organized in discrete generations.
131 Does were first mated at 18 weeks of age, thereafter 10 d after parturition. They
132 were under a constant photoperiod of 16:8 h and controlled ventilation. Animals
133 were fed with a standard commercial diet. A divergent selection experiment for
134 environmental variance of litter size variance was carried out over ten
135 generations. Each divergent line had approximately 125 females and 25 males
136 per generation. Selection was based on the phenotypic variance of litter size
137 within the female, after correcting litter size for both year-season and parity-
138 lactation status. As the genetic determination of all parities of a rabbit doe is
139 approximately the same and the permanent effects are the same along parities
140 (Piles *et al.,* 2006), after correcting for the systematic effects, the phenotypic
141 variance intra-doe records the environmental variability. All dams were ranked
142 based on their intra-doe variance of litter size. Only dams with four or more
143 parities were considered for selection. The best 20% dams were used to breed
144 the next generation. Each sire was mated with five dams and one male progeny
145 from the best dam that a sire was mated to was selected to breed the next
146 generation. This within-male family selection was performed in order to reduce
147 inbreeding. The experiment is described in Blasco *et al.* (2017)

148

149 *Blood collection*

One blood sample (3 mL) from the central ear artery was taken from 153 females of the 8$^{th}$ generation of selection at 18 weeks of age. Samples were collected into tubes containing K3-EDTA. Each sample was split into two aliquots. One aliquot was used to perform a hemogram. The other aliquot was centrifuged at 4,000 rpm for 15 min, in order to determine concentrations of C-reactive protein (CRP), complement C3, bilirubin, cholesterol and triglycerides. Immediately after this sampling, half of the females of both lines were vaccinated subcutaneously against viral haemorrhagic disease (CUNIPRAVAC® RHD), and the other half were vaccinated subcutaneously against myxomatosis (MIXOHIPRA® H). Three days after the vaccination, a blood sample was taken to perform a second hemogram and quantification of levels of CRP, complement C3, bilirubin, cholesterol and triglycerides. Five days after vaccination against viral haemorrhagic disease and myxomatosis, a subset of 29 vaccinated females of the high line and 25 vaccinated females of the low line was injected intramuscularly (i.m.) with 30 µg/kg of ACTH (ACTH 1-24, Sigma-Aldrich Co Ltd, Spain) at 8:00 a.m. A blood sample was collected to measure cortisol levels before and four hours after injection with ACTH. This time interval and concentration of ACTH 1-24 were established in according to previous study by Guelfi et al. (2011). For each assay, sample sizes were calculated considering half of the standard deviation of each trait as the relevant value for difference between lines. The samples were chosen at random within each group.

Resilience indicators

*Survival rate at birth and involuntary culling rate.* Survival rate at birth (SRB) was recorded in 3,589 deliveries of both divergent lines from the 8$^{th}$ to the 10$^{th}$ generation of selection (see Table 1); SRB was calculated as the ratio between the number of kits born alive and the total number of kits born. Involuntary culling rate (ICR) was estimated in both lines from the 8$^{th}$ to the 10$^{th}$ generation. Involuntary culling included deaths for unknown cause, disease occurrence and obstetric disorders (Table 2). Disease occurrence encompassed the cases of ulcerative pododermatitis, mastitis, abscesses, coryza and enteritis-diarrhoea. Obstetric disorders included the cases of death at delivery, mummified foetuses, prolapse and infertility. Elimination for infertility was considered when a doe had 4 consecutive non-fertile mating or 7 consecutive refusals to the buck.

*Hemogram.* White blood leukocyte count (WBC), and the percentage of lymphocytes and neutrophils were measured in 20 does of the high line and 20 does of the low line, before and after the vaccination with CUNIPRAVAC® RHD or MIXOHIPRA® H. All blood samples were collected in the same season. These parameters were assessed by ADVIA 120 Hematology Analyzer.

*Acute phase protein.* CRP plasma concentration was quantified in 74 does of the high line and 79 does of the low line, before and after the vaccination with CUNIPRAVAC® RHD or MIXOHIPRA® H, using a commercially available enzyme-linked immunoassay (ELISA) kit for rabbits (Life Diagnostics, Inc, PA, USA catalogue number 2210-5).

*Cortisol.* Cortisol plasma concentration was measured in 29 does of the high line

and 25 does of the low line, before and after the injection i.m. with 30 µg/kg of ACTH 1-24, using an ELISA *kit (Endocrine Technologies, Inc., CA, USA, catalogue number ERK R7003)*. Cortisol was measured instead of corticosterone because it is the predominant corticosteroid in rabbits' blood. The sensitivity of the assay was 0.15 ng/mL. The specificity of the assay was 100% for cortisol and <0.2% for corticosterone. The intra-assay and inter-assay coefficients of variation were 5.2% and 6.5% respectively.

*Biochemical parameters.* Plasma levels of complement C3, bilirubin, triglycerides and cholesterol were determined using Ecoline kits and automatic analyzer Microlab 300 (Merck®, Germany), spectrophotometer Genesys 10 (Thermo Fisher Scientific Inc., USA) and microprocessor-controlled analyzer EasyLite (Medica, Bedford, USA) according to manufacturer conditions. These biochemical parameters were measured in 10 does from the high line and in 10 does from the low line before and after vaccination with CUNIPRAVAC® RHD or MIXOHIPRA® H.

*Statistical analysis*

The correlated response in resilience indicators was estimated as the difference between the high and the low lines. Survival rate at birth was analysed using the following model:

$$y_{ijklmn} = \mu + YS_i + G_j + PL_k + L_l + b\ LS_{ijklmn} + p_{jlm} + e_{ijklmn}$$

Where $YS_i$ is the year-season effect with fifteen levels, $G_j$ is the generation with three levels ($8^{th}$, $9^{th}$ and $10^{th}$ generation of selection), $PL_k$ is the parity-lactation status effect with three levels (nulliparous, lactating and nonlactating female), $L_l$

224 is the line effect with two levels (high and low line), b is the regression coefficient,
225 $LS_{ijklmn}$ is the covariate litter size, $p_{jlm}$ is the dam permanent effect, and $e_{ijklmn}$ is
226 the residual term. Survival rate at birth was also analysed without the covariate
227 litter size in former model.
228 Involuntary culling rate was analysed using the following model:
229 $$y_{ijkl} = \mu + YS_i + G_j + L_k + e_{ijkl}$$
230 Where $YS_i$ is the year-season effect with fifteen levels, $G_j$ is the generation with
231 three levels, $L_k$ is the line effect with two levels and $e_{ijkl}$ is the residual term.
232 The differences between lines for white leukocyte count, lymphocytes,
233 neutrophils, and biochemical parameters before vaccination were analysed using
234 the following model:
235 $$y_{ijkl} = \mu + T_i + LT_j + p_{ijk} + e_{ijkl}$$
236 Where $T_i$ is the treatment effect with three levels (before vaccination, vaccination
237 with CUNIPRAVAC® RHD and MIXOHIPRA® H), $LT_j$ is the line-time effect with
238 four levels (the high line before vaccination, the low line before vaccination, the
239 high line at 3 day after vaccination and the low line at 3 day after vaccination), $p_{ijk}$
240 is the dam permanent effect and $e_{ijkl}$ is the residual term. The model for cortisol
241 and C-reactive protein also included a season effect, since there were several
242 batches throughout the year.
243
244 All analyses were performed using Bayesian methodology. Bounded uniform
245 priors were used for all effects with the exception of the dam permanent effect,
246 considered normally distributed with mean **0** and variance $\mathbf{I}\sigma_p^2$. Residuals were a
247 priori normally distributed with mean **0** and variance $\mathbf{I}\sigma_e^2$. The priors for the
248 variance were also bounded uniform. Features of the marginal posterior

distributions for all unknowns were estimated using Gibbs sampling. Inferences were derived from the marginal posterior distributions. Median, the highest posterior density region at 95% (HPD95%), and the guaranteed value at 80% of probability were provided (Blasco, 2017). Notice that in a Bayesian context there is no "significance", but the actual probabilities of the differences being higher or lower than zero are estimated instead. We consider that there is enough evidence about the high and the low line being different if the probability of $|D_{H-L}|$ is more than 0.90. The Rabbit program developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for all procedures (more details about its features are given at http://www.dcam.upv.es/dcia/ablasco/Programas/THE PROGRAM Rabbit.pdf). We used a chain of 60,000 samples, with a burn-in period of 10,000. Only one out of every 10 samples was saved for inferences. Convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures.

## Results

*Survival rate at birth and involuntary culling rate*

Table 3 shows descriptive statistics and the features of the estimated marginal posterior distributions of the differences between lines ($D_{H-L}$) for survival rate at birth and involuntary culling rate, and Table 2 gives information about the main causes for involuntary culling in these lines. The high line showed a higher perinatal mortality than the low line, before (P=1.00) and after correction for the covariate litter size (P=1.00). In a Bayesian context, several confidence intervals can be easily estimated. We can provide intervals [$k$, $+\infty$), where $k$ can be interpreted as

a guaranteed value with a determined probability (Blasco, 2017). We considered a guaranteed value at 80%. In this context, we note that the difference between the high and low lines for perinatal mortality was at least +2.9% (before correction for litter size) and +2.3% (after correction for litter size) with 80% probability. Moreover, the involuntary culling rate was also higher in the high line than in the low line; the difference between both lines was +12.4% (P=1.00) with a guaranteed value of +10.4%. Table 2 shows as the main causes of involuntary culling in our lines can be grouped into three groups; death (49%), disease occurrence (29%) and obstetric disorders (22%). Disease emergence is associated with several pathologies like ulcerative pododermatitis, mastitis, abscesses, coryza and enteritis-diarrhoea; while obstetric disorders are related to dam's reproductive problems, including postpartum complication, mummified foetuses, and infertility. Death rate and culling by disease were similar in both lines (47% in the high line vs 50% in the low line for death rate, and 27% in the high line vs. 31% in the low line for disease occurrence). However, incidence of reproductive problems was higher in the high line than in the low one (26% vs. 19%, respectively).

*Blood Parameters*

Tables 4 and 5 display the differences between lines ($D_{H-L}$) for several immunological and biochemical parameters, before and after stimulation by vaccination against myxomatosis or RHD viruses. Before vaccination, no difference in white blood leukocyte count (WBC) was found between lines (P<0.90), but the high line showed higher percentage of lymphocytes (+4.5%, P=0.95) and lower percentage of neutrophils (-4.0%, P=0.93) than the low line. Concentration of C-

reactive protein (CRP) and complement C3 were also higher in this line than in the low one (+5.6 µg/mL and +4.6 mg/mL, with P > 0.90). This is in accordance with a higher basal immune response in this line. We also found higher basal concentration in the high line than the low line for serum bilirubin (+7.9 mmol/L, P=0.93), triglycerides (+0.3 mmol/L, P=1.00) and cholesterol (+0.4 mmol/L, P=0.99). Immunological and biochemical responses were similar for both vaccines. After vaccination, the low line showed higher percentage of lymphocytes and CRP plasma concentration than the high one (+4.0% with P=0.93 for lymphocytes, and +13.1 µg/mL with P=1.00 for CRP); since increment was greater in this line (+7.5% vs -1.0% with P=1.00 for lymphocytes, and +29.3 µg/mL vs +10.7 µg/mL with P=1.00 for CRP). No difference in complement C3 was found between lines after vaccination. The difference between lines disappeared for serum bilirubin concentration, due to a larger increment in the low line than in the high line (+14.2 mmol/L vs +8.7 mmol/L, P=0.93). Besides, the low line increased slightly its levels of triglycerides, reducing the difference between lines (+0.11 mmol/L vs. +0.01, P=0.94). The difference in cholesterol was maintained between lines.

Table 4 also shows the levels of cortisol, before and after stimulation by ACTH. The high line had higher baseline cortisol concentration than the low line before stimulation by ACTH (+0.2 ng/mL, P=0.90). The difference between both lines increased more than three-fold after the injection of ACTH (P=1.00), because of a higher response to stimulation by ACTH in the high line than in the low one (+0.9 ng/mL vs. +0.4 ng/mL, P=0.99).

## Discussion

We have divergently selected two rabbit lines for environmental variance of litter size over ten generations, after which the line selected for variability of litter size showed greater variability (1.7 kits$^2$ more) and lower litter size (almost 1 kit less) than the homogeneous line (Blasco *et al.,* 2017), as a consequence of a lower early embryo development (García *et al.,* 2016) and lesser number of implanted embryos (Argente *et al.,* 2017) in this line. This divergence in litter size variability can be related to the dam's different capacity of adaptation to adverse environmental changes, which would imply difference in resilience between both lines. Resilience has an important role in animal's health and welfare. In this study, we found differences between lines in involuntary culling rate related to reproductive problems, and perinatal mortality. Moreover, García *et al.* (2018) observed in our lines that selection to increase litter size variability decreases body reserves at parturition. During delivery, there is a great energy mobilization in rabbit females. If the dam does not recover its body reserves after delivery, this leads to poor fertility (Fortun-Lamothe, 2006) and lowering of immunity defences (Castellini et al., 2010), which agrees with the larger rate of mortality at delivery and the higher elimination rate by infertility found in our heterogeneous line (16.8% and 8.2%) than in the homogenous one (13.3% and 3.7%). Therefore, increasing litter size variability can compromise dam's survival in the farm.

In order to understand the physiological bases of resilience in both divergent lines, we assessed specific biomarkers of diseases and stress, such as CRP, complement C3, concentration of lymphocytes and neutrophils in blood, and cortisol. C-reactive protein (CRP) is an acute phase protein secreted mainly by hepatic cells in response to inflammatory stimulus, activating the complement

system and the phagocytes (Rosa Neto and Carvalho, 2009). The complement C3 plays a central role in the complement system, attracting phagocytes to the inflammation site (Volanakis, 1990). Before vaccination, the high line presented higher levels of CRP and complement C3 than the low line. A higher concentration of CRP and complement C3 are related to a higher sensitivity to diseases (Markanday, 2015). Besides, neutrophils provide the first-line defence against infection in innate immune response. Mature neutrophils are rapidly mobilized from the bone marrow reserve during an inflammatory episode or in response to infection, resulting in a dramatic rise in circulating neutrophil numbers within a matter of hours (Furze and Rankin, 2008). A higher number of neutrophils in the low line can help the rapid elimination of pathogens and would lead to inactivate the infection. If infection persists, lymphocytes will be involved in the activation of the adaptive immune response (Janeway *et al.,* 2001). Therefore, a lower concentration of neutrophils and a higher concentration of lymphocytes in the high line would show a weaker defend barrier and a greater susceptibility to infections in this line, corroborating a higher sensitivity to common microorganisms in the farm.

Vaccination represents a useful model for studying the dynamics of inflammatory response against pathogen exposure, with the advantage of delivering a controlled dose of antigen (Posthouwer et al., 2004). In this study, we used two types of vaccines: a live attenuated vaccine (as MIXOHIPRA® H) and an inactivated vaccine (as CUNIPRAVAC® RHD). Live attenuated vaccines can induce a greater immune response than inactivated vaccines. In order to increase this immunity response, inactivated vaccines include adjuvants that increase

antibody production and can cause a slightly greater level of inflammation. This explains the similar response in immunological and biochemical blood parameters that we found between both types of vaccines. After vaccination, the low line showed a higher increase in concentration of CRP and in lymphocytes than the high line. These findings agree with a quicker and higher inflammatory response in this line. McDade et al. (2015) studied the inflammatory response to influenza vaccination. These authors suggest that the combination of a low baseline CRP concentration and a robust increase in response to challenge of vaccination is related to a good functionality of the inflammatory system. This would agree with a lower risk for disease, supporting a higher resistance to disease in the low line.

Infectious and inflammatory processes induce alterations in the liver metabolism, leading to reducing inflammation and fighting infections. When an infection takes place, the most common change is a decrease in cholesterol, an increase in plasma triglycerides and an increase in secretion of hepatic VLDL (very low-density lipoprotein) (Feingold and Grunfeld, 2015). Inflammation and infections can also increase bilirubin levels (Minemura *et al.,* 2014). We found higher basal concentrations of serum bilirubin, cholesterol and triglycerides in the high line than in the low line. A higher concentration of bilirubin and triglycerides in the high line would be related to a larger inflammatory response in this line, due to higher sensibility to diseases. The changes in bilirubin, lipids and lipoproteins during inflammation and infection are part of the innate immune response, therefore they are likely playing an important role in protecting the host. In this sense, bilirubin is a natural antioxidant and it has been postulated as one of the principal protective

399 mechanisms against oxidative stress and inflammatory processes (Otero *et al.,*
400 2009). In relation to cholesterol, De Nardo *et al.* (2014) identified that
401 antiinflammatory effects of high-density lipoprotein (HDL) are mediated through the
402 induction of ATF3. The ATF3 is a key transcriptional regulator of innate immune
403 response genes, which is induced by Toll-like receptor (TLR) stimulation and acts
404 as negative regulator of proinflammatory cytokines. Besides, Barcia and Harris
405 (2005) found that triglycerides are involved in both attenuation of the response of
406 hepatocytes to circulating proinflammatory cytokines, thereby down-regulating the
407 overall acute phase response and activating neutrophils. The response after the
408 vaccine was higher in the low line for serum bilirubin (+14.2 mmol/L) and
409 triglycerides (+0.11 mmol/L) than in the high one (+8.7 mmol/L for bilirubin and
410 +0.01 mmol/L for triglycerides), these results agree with the protective role of
411 bilirubin and triglycerides against the quicker inflammatory response found in the
412 low line.
413
414 In relation to sensibility to stress, it is well documented that a stressful stimulus
415 actives the HPA axis, releasing ACTH. Secretion of ACTH acts on adrenocortical
416 cells to initiate synthesis and release of glucocorticoids (Janssens *et al.,* 1995).
417 Thus, baseline levels of cortisol provide insight into the stress state in animals
418 (Cabezas *et al.,* 2007). In this regard, the higher basal cortisol concentration in
419 the high line would be related to a higher chronic stress. On the other hand, it
420 was reported that the sensitivity of the adrenocortex to circulating ACTH
421 increases after chronic stress (Janssens *et al.,* 1995). The higher increase in
422 cortisol in response to ACTH in the high line would agree with a higher
423 susceptibility to stress in this line. A larger sensibility to stress or poor coping with

environmental perturbations leads to lower immune competence and higher disease susceptibility (Glaser and Kiecolt-Glaser, 2005). CRP and complement C3 are used as biomarkers of inflammation and infection (Eckersall, 2000; Volanakis, 1990). In agreement to a greater chronic stress and larger susceptibility to stress in the high line, we found higher inflammatory response in this line, i.e. higher levels of CRP and complement C3. Moreover, the negative effect of stress on reproduction by both poor oocyte quality and delayed embryonic development has been widely described (Burkuš *et al.,* 2015). The high line showed a smaller embryonic development and a lower number of implanted embryos than the low line (García *et al.,* 2016; Argente *et al.,* 2017). These differences in reproductive fitness can be related to differences in the corticotrope response to stress and to differences in inflammatory response, which are two important components of physiological adaptation to environmental challenges, i.e. to resilience.

## Conclusions

Selection for greater litter size variability shows a negative correlated response in survival rate at birth and involuntary culling rate, which can be related with susceptibility to disease and stress; i.e. with resilience. Therefore, selection for litter size variability leads to a correlated response in dam's resilience.

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## Declaration of interest

The authors declare that they have no competing interests.

## Ethics statement

All experimental procedures involving animals were approved by the Miguel Hernández University of Elche Research Ethics Committee (Reference number UMH.DTA.MJA.01.14), in accordance with Council Directives 98/58/EC and 2010/63/EU.

## Software and data repository resources

Data and software are available upon request to the corresponding author.

**Table 1** *Number of females and deliveries in the high (H) and the low (L) line from the 8th to the 10th generation.*

| Generation | 8th | | 9th | | 10th | | |
|---|---|---|---|---|---|---|---|
| | H | L | H | L | H | L | Total |
| Females | 123 | 152 | 166 | 173 | 161 | 163 | 938 |
| Deliveries | 433 | 485 | 581 | 569 | 827 | 694 | 3,589 |

467 **Table 2** *Number of involuntary culling females in the high and the low lines* from
468 the 8th to the 10th generation.

| | high line | low line |
|---|---|---|
| *1. Death for unknown cause* | 137 | 148 |
| Sub-total | 137 (47%) | 148 (50%) |
| *2. Disease occurrence* | | |
| Ulcerative pododermatitis | 34 | 41 |
| Mastitis | 25 | 30 |
| Abscesses | 9 | 8 |
| Coryza | 8 | 7 |
| Enteritis-diarrhoea | 3 | 5 |
| Sub-total | 79 (27%) | 91 (31%) |
| *3. Obstetric disorders* | | |
| Death at delivery | 49 | 39 |
| Mummified foetus | 1 | 3 |
| Prolapse | 2 | 2 |
| Infertility[a] | 24 | 11 |
| Sub-total | 76 (26%) | 55 (19%) |
| *Total* | *292* | *294* |

469 a: does were culled for infertility after 4 consecutive non-fertile matings or with 7
470 consecutive refusals to the buck.

471

**Table 3** *Correlated response to selection for environmental variability of litter size in kits survival rate at birth before (SRB) and after correction by litter size ($SRB_{LS}$), and in female involuntary culling rate (ICR).*

| | $n_H$ | H | $n_L$ | L | $D_{H-L}$ | $HPD_{95\%}$ | P | $k_{80\%}$ |
|---|---|---|---|---|---|---|---|---|
| SRB, % | 1841 | 81.1 | 1748 | 85.1 | -4.1 | -6.4, -1.9 | 1.00 | -2.9 |
| $SRB_{LS}$, % | 1841 | 81.9 | 1748 | 85.1 | -3.2 | -5.4, -1.1 | 1.00 | -2.3 |
| ICR, % | 450 | 60.3 | 485 | 47.9 | 12.4 | 7.8, 16.8 | 1.00 | 10.4 |

$n_H$: total number of kits born in the high line. $n_L$: total number of kits born in the low line. H: mean of the high line. L: mean of the low line. $D_{H-L}$: difference between the high and the low lines. $HPD_{95\%}$: highest posterior density region at 95%. P: probability of the difference being > 0 when $D_{H-L} > 0$ or being < 0 when $D_{H-L} < 0$. $k_{80\%}$: guaranteed value at 80%, limit of the interval $[k, +\infty)$ when $D_{H-L} > 0$ and $(-\infty, k]$ when $D_{H-L} < 0$ at 80% of probability.

486 **Table 4** *Correlated response to selection for environmental variability of litter size in immune parameters and cortisol.*

| | | $n_H$ | H | $n_L$ | L | $D_{H-L}$ | $HPD_{95\%}$ | P | $k_{80\%}$ |
|---|---|---|---|---|---|---|---|---|---|
| WBC (x$10^3$/µL) | $Before_{vaccine.}$ | 20 | 9.3 | 20 | 8.9 | 0.4 | -1.0, 1.8 | 0.69 | - |
| | $After_{vaccine.}$ | 20 | 9.4 | 20 | 9.6 | -0.2 | -1.6, 1.2 | 0.60 | - |
| Lymphocytes (%) | $Before_{vaccine.}$ | 20 | 66.9 | 20 | 62.4 | 4.5 | -0.7, 10.1 | 0.95 | 2.3 |
| | $After_{vaccine.}$ | 20 | 65.9 | 20 | 69.9 | -4.0 | -9.8, 1.1 | 0.93 | -1.7 |
| Neutrophils (%) | $Before_{vaccine.}$ | 20 | 23.7 | 20 | 27.8 | -4.1 | -9.1, 0.9 | 0.95 | -2.0 |
| | $After_{vaccine.}$ | 20 | 24.0 | 20 | 22.2 | 1.8 | -3.2, 7.3 | 0.76 | - |
| Complement C3, mg/mL | $Before_{vaccine.}$ | 10 | 14.1 | 10 | 9.5 | 4.6 | -2.1, 12.2 | 0.90 | 1.3 |
| | $After_{vaccine.}$ | 10 | 13.7 | 10 | 9.9 | 3.8 | -8.9, 11.7 | 0.79 | - |
| CRP, µg/mL | $Before_{vaccine.}$ | 74 | 32.1 | 79 | 26.6 | 5.6 | -3.4, 14.3 | 0.91 | 1.7 |
| | $After_{vaccine.}$ | 74 | 42.8 | 79 | 55.9 | -13.1 | -22.4, -4.3 | 1.00 | -10.5 |
| Cortisol, ng/mL | $Before_{ACTH}$ | 29 | 0.8 | 25 | 0.6 | 0.2 | -0.1, 0.4 | 0.90 | 0.1 |
| | $After_{ACTH}$ | 29 | 1.7 | 25 | 1.0 | 0.7 | 0.4, 0.9 | 1.00 | 0.5 |

$n_H$: number of data in the high line. $n_L$: number of data in the low line. H: mean of the high line. L: mean of the low line. $D_{H-L}$: differences between the high and low lines at $8^{th}$ generation. $HPD_{95\%}$: highest posterior density region at 95%. P: probability of the difference being > 0 when $D_{H-L} > 0$ or being < 0 when $D_{H-L} < 0$. $k_{80\%}$: guaranteed value at 80%, limit of the interval $[k, +\infty)$ when $D_{H-L} > 0$ and $(-\infty, k]$ when $D_{H-L} < 0$ at 80% of probability. WBC: white blood cells. CRP: C-reactive protein.

**Table 5** *Correlated response to selection for environmental variability of litter size in biochemical parameters related to liver functionality.*

| | | $n_H$ | H | $n_L$ | L | $D_{H-L}$ | $HPD_{95\%}$ | P | $k_{80\%}$ |
|---|---|---|---|---|---|---|---|---|---|
| Bilirubin, mmol/L | $Before_{vaccine.}$ | 10 | 21.2 | 10 | 13.4 | 7.9 | -2.8, 17.9 | 0.93 | 3.4 |
| | $After_{vaccine.}$ | 10 | 29.9 | 10 | 27.6 | 2.2 | -7.7, 12.9 | 0.68 | - |
| Triglycerides, mmol/L | $Before_{vaccine.}$ | 10 | 0.9 | 10 | 0.6 | 0.3 | 0.1, 0.5 | 1.00 | 0.2 |
| | $After_{vaccine.}$ | 10 | 0.9 | 10 | 0.7 | 0.2 | -0.1, 0.4 | 0.96 | 0.1 |
| Cholesterol, mmol/L | $Before_{vaccine.}$ | 10 | 2.5 | 10 | 2.1 | 0.4 | 0.1, 0.8 | 0.99 | 0.3 |
| | $After_{vaccine.}$ | 10 | 2.7 | 10 | 2.1 | 0.6 | 0.2, 1.1 | 1.00 | 0.4 |

$n_H$: number of data in the high line. $n_L$: number of data in the low line. H: mean of the high line. L: mean of the low line. $D_{H-L}$: differences between the high and low lines at $8^{th}$ generation. $HPD_{95\%}$: highest posterior density region at 95%. P: probability of the difference being > 0 when $D_{H-L} > 0$ or being < 0 when $D_{H-L} < 0$. $k_{80\%}$: guaranteed value at 80%, limit of the interval $[k, +\infty)$ when $D_{H-L} > 0$ and $(-\infty, k]$ when $D_{H-L} < 0$ at 80% of probability.

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