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Moreau, J., Martinaud, G., Troussard, J.-P., Zanchi, C. and Moret, YT. 2012. Trans-generational immune priming is constrained by the maternal immune response in an insect. – Oikos 121: 1828-1838.

Appendix 1

This supplementary reports the result of a preliminary study investigating variation of egg volume as a function of maternal immune challenge and mother body size.

Methods

Age controlled virgin beetles (8 days ± 1 day post emergence) were obtained from pupae collected from stock cultures maintained at 25°C with ad libitum supply of food and water. Thirty-two females received a single injection of 5 µl of Ringer's solution containing non-purified lipopolysaccharides (LPS: 0.5 mg ml^{-1}) extracted from *Escherichia coli* (Sigma: L8274) and 30 females received a similar injection of Ringer's solution but without LPS as procedural control. All injections were made through the pleural membrane between the second and the third abdominal tergites, using sterilized pulled glass capillaries after immobilisation of the insects on ice for 10 min.

Immediately after their immune treatment, females were paired with a virgin and unchallenged male for four days in a petri dish provided with bleach flour and ad libitum food and water under standard laboratory conditions (25°C, 70% RH, L12h:D12h). On the 4th day post maternal challenge, eggs were searched by sieving the flour (Ø 600 µm) of the petri dish. Four random eggs per female were individually collected and photographed using a camera fitted on a binocular microscope (magnification x 10) and measured for their length (L in mm) and their width (W in mm). Since the shape of a beetle egg is ellipsoidal, the volume of each egg was calculated following the equation: Egg volume (in mm^3) = $4/3*\pi*(W/2)^2*(L/2)$. Females were used to estimate their body size by measuring the length of the left elytra with a Mitutuyo digital calliper (precision ± 0.02 mm).

Variation in volume of the eggs was analysed using an ANCOVA with mother immune challenge as fixed factor and mother body size as covariate.

Results and conclusions

Female body size ranged from 7.82 mm for the smallest and 11.32 mm for the largest (mean ± SE = 9.65 ± 0.09 mm, median = 9.83 mm) and the mean size of their eggs ranged from 0.39 mm^3 for the smallest and 0.72 mm^3 for the largest (mean ± SE = 0.56 ± 0.01 mm^3, median = 0.56 mm^3). Egg volume was not significantly explained by the immune challenge of the mothers and their body size, either as main effect or interaction term (Table A1). Therefore, the relationship between antibacterial activity of the eggs and that of the haemolymph of small immune-challenged females (Fig. 3) is unlikely confounded by egg size.

Table A1. Results of the ANCOVA examining variation of egg volume (mm^3) as a function of the maternal immune challenge (Challenge – fixed factor) and mother body size (Size - covariate).

Source	F	DF	p
Global model	1.82	3,58	0.154
Challenge	0.18	1,58	0.669
Size	2.71	1,58	0.105
Size × Challenge	0.106	1,58	0.746