

In search of genetic constraints limiting the evolution of egg size: Direct and correlated responses to artificial selection on a prenatal maternal effector

Pick, J.L., Hutter, P., & Tschirren, B

Supplementary Materials

S1 Relative and absolute egg size

Relative egg size was highly correlated with absolute egg size in all lines, replicates and generations (mean $r \pm \text{SD}$, 0.884 ± 0.120 , $n=14$). We tested whether the strength of this correlation changed over the course of the experiment, by regressing correlation coefficients against generation. The correlation between absolute and relative egg size did not change over the course of the experiment ($F_{1,12} = 0.20$, $P = 0.661$; Figure S1).

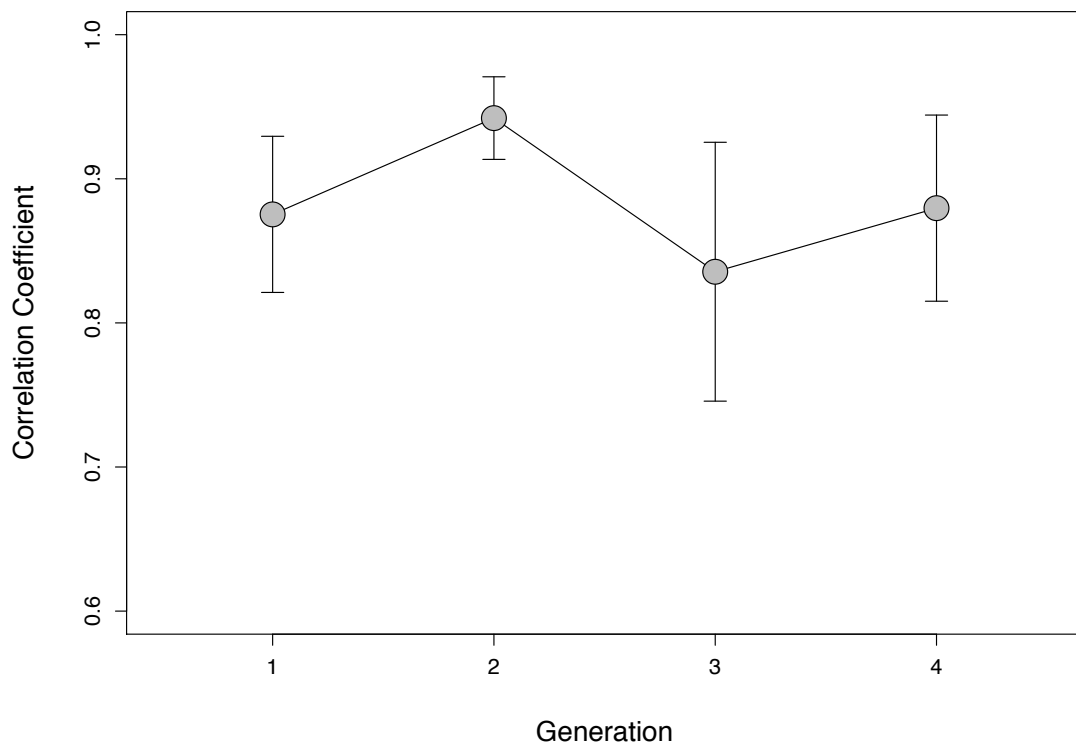


Figure S1: Correlation coefficients between relative and absolute egg size across generations.

S2 Unselected base population

Alongside the selection lines, an unselected base population was maintained in the same facility, originating from the same founder populations. These birds were not bred at the same times or ages as the selection lines and so are not directly comparable. Nevertheless, given that there was no directional change in mean egg size over 5 generations in this unselected population ($F_{1,3} = 0.51$, $P = 0.528$; see Figure S2), we can exclude the possibility that systematic changes in egg size have occurred over time due to inadvertent effects of husbandry.

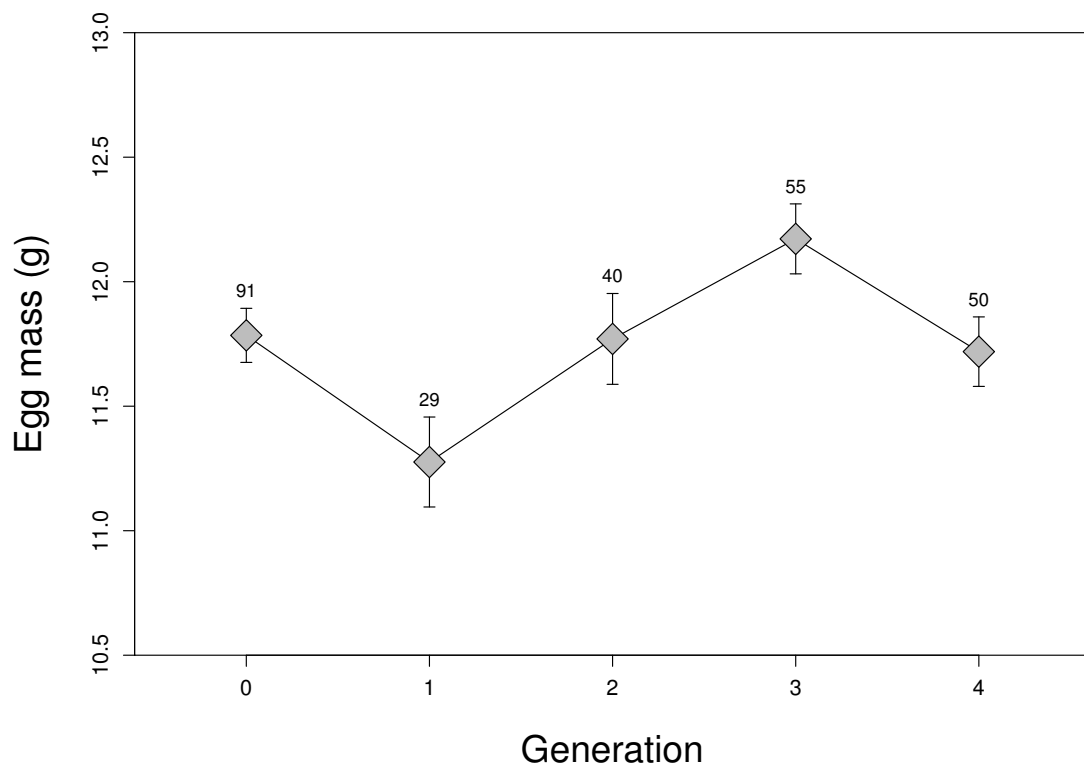


Figure S2: Egg size in the unselected base population across generations. Egg size fluctuates randomly between generations, but not in a directional manner across 5 generations. The number above each point represents sample size. Note that these females were bred at a different time to the two selection line replicates, and so cannot be used as a direct control.

S3 Changes in egg traits over the course of the laying sequence

We measured the repeatability of egg size and components in generation 4, using eggs collected on days 1, 2, 15 and 16 of egg collection (80 females, 2-4 eggs per female). Egg size and all egg components were highly repeatable within females (Table S1).

Table S1: Repeatabilities of egg traits of the dissected and dried eggs in generation 4 of the selection lines.

Trait	$r \pm \text{SE}$	$F_{79,204}$	P
Egg Size	0.855 ± 0.024	21.92	< 0.001
Dry Mass	0.822 ± 0.029	17.38	< 0.001
Wet Albumen	0.879 ± 0.021	26.72	< 0.001
Dry Albumen	0.871 ± 0.022	24.91	< 0.001
Wet Yolk	0.801 ± 0.032	15.25	< 0.001
Dry Yolk	0.798 ± 0.032	15.06	< 0.001
Dry Shell	0.811 ± 0.031	16.23	< 0.001

In order to test whether egg traits change over the course of the laying sequence, we compared eggs laid at the beginning and end of the laying sequence using linear mixed effects models. Within-individual means of eggs measured at the beginning and end of the sequence were used in the analysis, and only individuals that had eggs measured at both time points were included in the analysis ($n = 77$ females). Timepoint, line and replicate were included as fixed factors, as well as the interaction between timepoint and line (indicating a line specific change in egg components). Female ID was included as a random effect. Significance was determined using likelihood ratio tests between nested models, meaning degrees of freedom in all comparisons was 1.

Dry albumen mass significantly increased over the laying sequence (Table S2, Figure S3). The effect of selection line on egg mass and wet albumen mass became

more pronounced later in the laying sequence (Table S2, Figure S3). Total dry mass, wet yolk, dry yolk and dry shell masses all did not change over the laying sequence (Table S2), although there was a tendency for the selection line effect presented in the results to become more pronounced later in the laying sequence (Table S2).

Table S2: Difference between egg traits at the beginning and end of a laying sequence.

Trait	Line		Timepoint		Line x Timepoint		Replicate	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Egg Size	-	-	-	-	5.11	0.024	1.15	0.283
Dry Mass	26.10	<0.001	0.13	0.716	3.45	0.063	3.47	0.062
Wet Albumen	-	-	-	-	6.04	0.014	0.00	0.956
Dry Albumen	35.39	<0.001	8.10	0.004	2.58	0.108	0.39	0.532
Wet Yolk	12.54	<0.001	0.12	0.726	2.97	0.085	4.85	0.028
Dry Yolk	12.22	<0.001	0.11	0.739	2.91	0.088	4.11	0.043
Dry Shell	29.15	<0.001	2.12	0.145	2.75	0.097	1.67	0.197

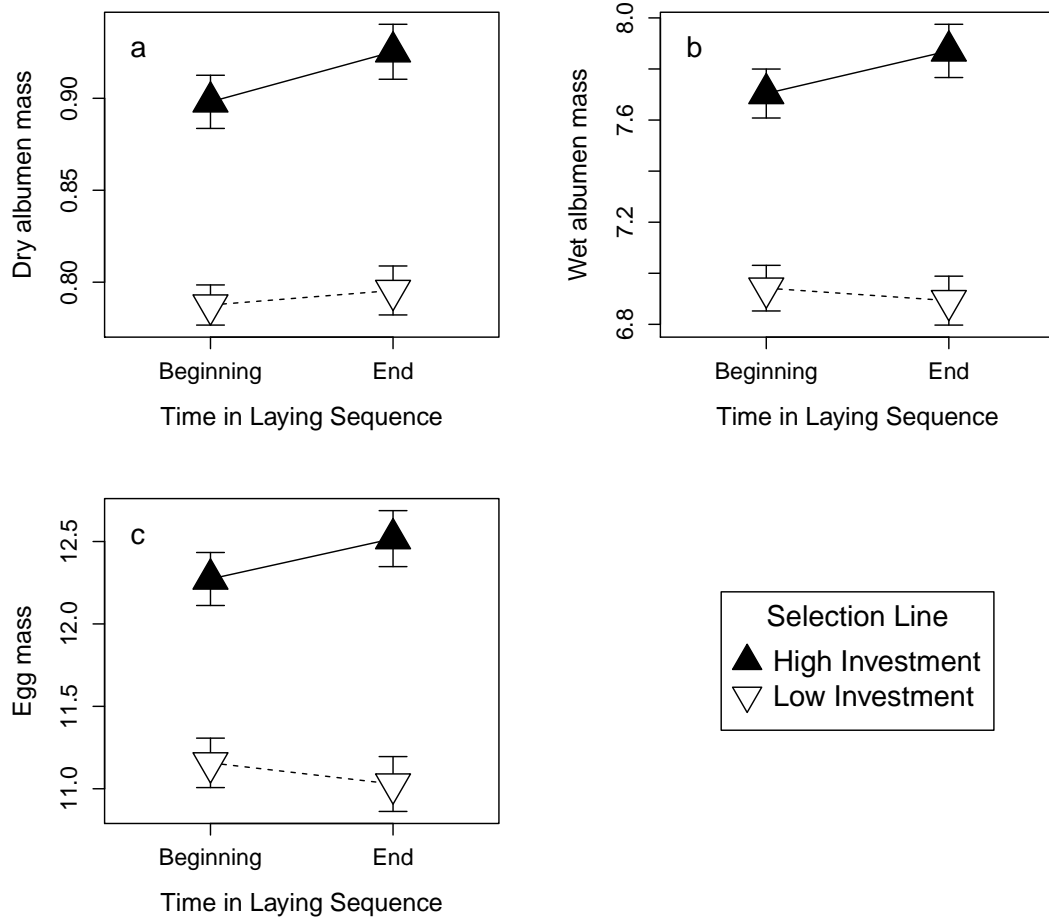


Figure S3: Difference between a) Dry Albumen Mass, b) Wet albumen mass and c) Egg mass at the beginning and end of a measured laying sequence.

S4 Difference in egg size between selection lines

Table S3: Difference in egg size between high and low investment lines across generations and replicates.

Generation	Replicate	Difference (g)	<i>t</i>	df	<i>P</i>
2	1	0.58	2.15	38	0.038
3	1	0.85	2.58	38	0.014
4	1	1.21	3.91	38	0.000
5	1	1.79	5.62	28	0.000
2	2	0.58	2.17	38	0.037
3	2	0.89	3.04	37	0.004
4	2	1.48	5.39	38	0.000
5	2	1.34	3.27	29	0.003