

Supplementary information

Joron M & PM Brakefield (2003). Captivity masks inbreeding effects on male mating success in butterflies. *Nature* 424: 191-194.

- A. Pilot experiments: effect of dust-colour and efficiency of transfer in copulation.
- B. Results of a greenhouse experiment using inbred ($F=0.25$) females.
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A. Pilot experiments: effect of dust-colour and efficiency of transfer in copulation.

3-5-day-old males from the outbred stock were divided at random into two cohorts, each of 51 individuals. These were then treated with orange and yellow dusts, respectively. The 102 males were released into a mesh flight cage of $2.45 \times 0.85 \times 0.85 \text{ m}^3$ ($l \times w \times h$) with a base of leaves and compost together with four food sources of banana. The cage was within the same climate room used for the cage mating trials. Sixty virgin, undusted, outbred females (3-4-day-old) were released a day later. Fifty-one females mated (85%) over the following six hours and were removed carefully as mating occurred; males were returned to the cage after copulation. 25 females mated with orange-dusted, and 26 with yellow-dusted males ($G_{df=1}=0.02$, $P=0.88$). All fifty-one females showed clear transfer of fluorescent dust during copulation. Thus in these conditions of an intermediate freedom of flight, mating success was independent of dust-colour and there was a 100% efficiency of dust-transfer during copulation.

B. Results of a greenhouse experiment using inbred females

We performed an additional greenhouse experiment to explore whether female inbreeding may affect male mating success, by releasing inbred ($F=0.25$) virgin females to be mated by the previously released F_0 and $F_{0.25}$ males. Inbred males had a mating success 69% that of outbred males (Table S1). Although the difference is not significant, the results are fully

consistent with those of the other experiments which all used outbred ($F=0$) females, suggesting female inbreeding has no important effect on male mating success.

Table S1. Inbred female mating and male recapture in greenhouse experiment VI

Females ($F_{0.25}$):	Released:	91
	Recaptured (%):	72.5
	Mated with: F_0	35
	$F_{0.25}$	23
	$F_0+F_{0.25}$	7
	unmated	1
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Males:	Released ($F_0+F_{0.25}$)	65+65
	Recaptured (%)	68.5
	F_0 (green)	46
	$F_{0.25}$ (orange)	43

C. Non-significance of line effect in laboratory trials

We tested for a line effect on the probability of mating in the laboratory cage trials by means of a mixed General Linear Model. For each male, the response variable was a binary response (1=did mate, 0=did not mate), with the inbreeding coefficient (IC) as a fixed factor, and line number as a random factor (nested in IC). Only successful trials involving a total of 40 lines were analysed (one $F_{0.25}$ line was involved in only trials without matings). There was no significant effect of male line on the probability of mating (Table S2). Inter-line variability in mating success is, therefore, unlikely to have played an important role in our experiments.

Table S2. Summary of GLM analysis for line effect

Source	DF	Seq SS	Adj SS	Adj MS	F	P
IC	2	1.8919	1.4888	0.7444	3.01	0.052
LINE(IC)	37	10.9252	10.9252	0.2953	1.37	0.075
Error	515	110.8478	110.8478	0.2152		
Total	554	123.6649				

IC, the level of inbreeding, is a fixed factor with 3 levels, 0, 0.25 and 0.375. LINE, the line number, is a random factor nested in IC. DF=degrees of freedom, Seq SS=sequential sum of squares, Adj SS=adjusted sum of squares, Adj MS=adjusted mean squares.

D. Absence of effect of male size on mating success in laboratory trials

An index of male size was measured after the two laboratory experiments using the distance between the foci of the two dorsal forewing eyespots²⁰ (Interfocal Distance, IFD, which is ca 0.3 of forewing length). Male size is significantly affected by inbreeding coefficient (ANOVA: $F_{2,519}=4.25$, $P=0.015$), with outbred males being slightly larger than inbred males (Table S3). However, neither IFD nor its interaction with inbreeding coefficient (IC) had a significant effect on mating success (tested by means of a binary logistic regression -- Table S4). The effect of within-trial relative size (Rank) was also non significant (Table S5; see also ref. 20).

Table S3. Size of successful and unsuccessful males in laboratory trials

	IC	Mating (N=174*)	Not mating (N=348)	Total (N=522)
Mean IFD	F ₀	60.25 (3.09)	60.73 (3.60)	60.53 (3.39)
	F _{0.25}	60.29 (3.11)	59.30 (3.73)	59.57 (3.59)
	F _{0.375}	59.68 (4.28)	59.33 (4.50)	59.44 (4.43)
	Overall	60.09 (3.47)	59.74 (4.00)	59.86 (3.83)
Mean Rank	F ₀	1.75 (0.71)	1.80 (0.79)	1.78 (0.76)
	F _{0.25}	2.02 (0.81)	2.14 (0.87)	2.11 (0.85)
	F _{0.375}	2.06 (0.77)	2.14 (0.82)	2.11 (0.80)
	Overall	1.92 (0.77)	2.04 (0.84)	2.00 (0.82)

IFD is given in millimetres x 10. Relative size is the rank among the three males in each trial (1>2>3). Standard deviations are given in parentheses. *, The butterflies used in the first 11 trials performed could not be measured

Table S4. Summary of binary logistic regressions for an effect of size in laboratory trials

Predictor	Coefficient	s.e.	Z	P	Odds Ratio	95% CI	
						Lower	Upper
Effect of absolute size							
Constant	2.207	2.708	0.82	0.415			
IC							
0.250	-7.897	3.963	-1.99	0.046	0.00	0.00	0.88
0.375	-4.074	3.552	-1.15	0.251	0.02	0.00	17.94
IFD	-0.01051	0.01118	-0.94	0.347	0.99	0.97	1.01
IC x IFD							
0.250	0.03031	0.01642	1.85	0.065	1.03	1.00	1.06
0.375	0.01509	0.01475	1.02	0.306	1.02	0.99	1.04
Effect of relative size							
Constant	-0.2429	0.4125	-0.59	0.556			
IC							
0.250	0.4326	0.6264	-0.69	0.490	0.65	0.19	2.21
0.375	-0.2540	0.6321	-0.40	0.688	0.78	0.22	2.68
Rank	-0.0915	0.2144	-0.43	0.669	0.91	0.60	1.39
IC x Rank							
0.250	-0.0679	0.3014	-0.23	0.822	0.93	0.52	1.69
0.375	-0.0341	0.3032	-0.11	0.910	0.97	0.53	1.75

E. Duration of mating.

During the laboratory experiment B, we recorded the time spent *in copula* for 104 pairings.

There is no significant effect of male inbreeding coefficient on copulation duration

(ANOVA: $F_{2,101}=1.56$, $P=0.215$). Range is 10mn to 120mn.

Table S5. Duration of mating of inbred and outbred males.

IC	N	Mean duration (in min)	s.d.
F ₀	45	29.78	20.11
F _{0.25}	30	27.33	12.44
F _{0.375}	29	36.38	26.76
Overall	104	30.91	20.58