Challenges in estimating heritability of phase polyphenism: insights from measured and simulated data in the desert locust -

Supporting Information

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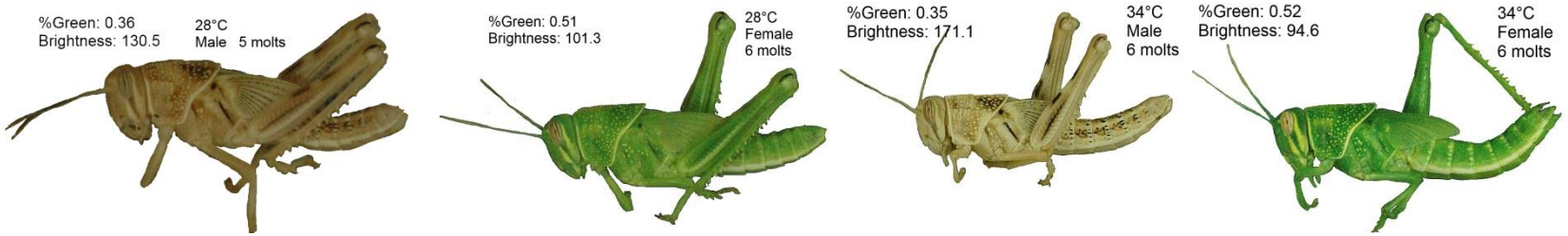
1- Details on methods and results from the biological data set on phase traits

1.1- Methods for larval pronotum color measurement

Last-instar larvae were anesthetized with CO_2 and photographed in lateral view. Pictures were corrected for white balance, using the PhotoFiltre 7© software improved by an external module (plugin "wbadjust"), and analyzed using ImageJ V1.37 (Abràmoff, Magalhães & Ram, 2004). Briefly, we selected the whole surface of the pronotum, using the "Polygone selection" function, and measured each color channel (RGB) as a 8–bit display mean value in the range 0–255, using the "Color Histogram" function. We then calculated the level of brightness as (R+G+B)/3 (Abràmoff, Magal hães & Ram, 2004) and the percentage of green color as G/(R+G+B). Brightness was transformed (1/x, based on Box-Cox normality plots) before analyses to ensure normality of the variable (Osborne, 2010).

Figure S1. Illustrations of larvae reared at 28°C (left) and 34°C (right), with the related percentage of green color and brightness measures.

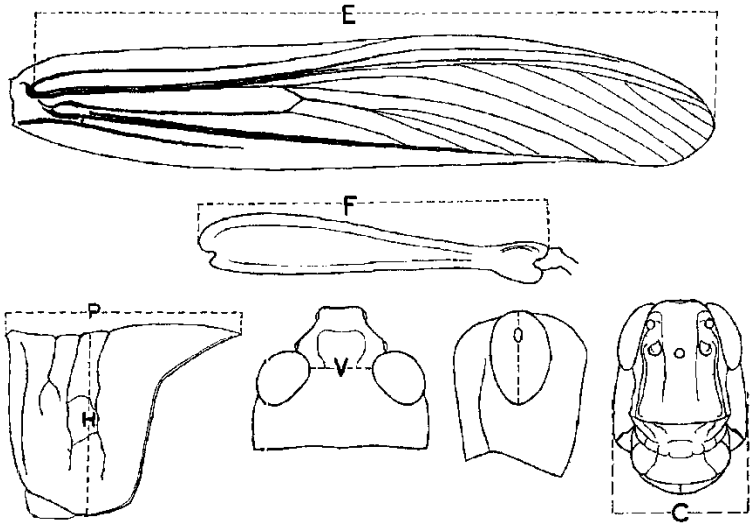


1.2- Methods for adult body shape measurement

Adults were anesthetized with CO_2 and photographed, along with a scale of 30mm, in both lateral and dorsal views. Pictures were analyzed with the software tpsDig© 2.17. We measured 7 morphometric distances in adult locusts: the elytron length (E), the maximum height of the pronotum (H), the length of the hind femur (F), and the vertical diameter of eyes (O), the pronotum length (P), the maximum width of the head (C), and the width of the vertex between eyes (V).

Figure S2. A. Illustrations of measurements used for calculating the 5 shape variables and the body size. B. Illustrations of an adult at time of morphometric measurements in lateral and dorsal views.

A



B

1.3- Means and standard deviations for phase variables as a function of extra-molting, sex and temperature

Table S1. Means and standard deviations for the eight phase traits measured on the desert locust

	♀ 28°C 5 molts	♀ 28°C 6 molts	♀ 34°C 5 molts	♀ 32°C 6 molts	♂ 28°C 5 molts	♂ 28°C 6 molts	♂ 32°C 5 molts	♂ 32°C 6 molts
N (coloration traits)	11	45	8	24	33	23	21	20
% Green	0.44 ± *0.03*	0.45 ± *0.03*	0.46 ± *0.02*	0.46 ± *0.03*	0.44 ± *0.04*	0.44 ± *0.03*	0.41 ± *0.04*	0.45 ± *0.04*
Brightness	120.54 ± *16.99*	114.68 ± *12.83*	127.05 ± *11.94*	131.86 ± *26.49*	116.94 ± *21.09*	128.58 ± *35.07*	139.88 ± *22.44*	129.99 ± *20.82*
N (morphological traits)	11	39	14	25	35	23	47	18
E/F	1.99 ± *0.07*	2.03 ± *0.06*	2.10 ± *0.08*	2.09 ± *0.07*	2.03 ± *0.06*	2.02 ± *0.10*	2.05 ± *0.07*	2.03 ± *0.06*
F/C	3.99 ± *0.19*	3.93 ± *0.22*	3.90 ± *0.23*	3.92 ± *0.16*	3.76 ± *0.17*	3.79 ± *0.25*	3.86 ± *0.22*	3.90 ± *0.17*
F/V	13.51 ± *0.65*	13.87 ± *2.08*	14.23 ± *1.46*	14.40 ± *1.43*	13.31 ± *1.57*	13.29 ± *1.78*	14.73 ± *1.72*	15.41 ± *1.59*
O/V	1.96 ± *0.12*	1.95 ± *0.31*	2.00 ± *0.23*	2.08 ± *0.23*	2.09 ± *0.29*	2.01 ± *0.28*	2.26 ± *0.26*	2.32 ± *0.23*
F	3.43 ± *0.10*	3.44 ± *0.11*	3.43 ± *0.15*	3.41 ± *0.10*	3.33 ± *0.09*	3.34 ± *0.12*	3.39 ± *0.12*	3.43 ± *0.08*
Max larval weight	1.87 ± *0.15*	2.28 ± *0.21*	1.95 ± *0.24*	2.32 ± *0.24*	1.30 ± *0.10*	1.53 ± *0.13*	1.27 ± *0.12*	1.49 ± *0.16*

N: Number of measured individuals for coloration and morphological traits; *Brightness*: Level of brightness, which is inversely related to the level of black pigmentation; *%Green*: Percentage of green color; *E*: Length of the fore wing; *F*: Length of the hind femur; *C*: Maximum width of the head; *H*: Height of the pronotum; *P*: Length of the pronotum; *O*: Vertical diameter of eyes; V: the width of the vertex between eyes.

1.4- Effects of extra-molting, sex and temperature on phase variables

We launched linear models in R 3.2.3 (R Core Team, 2015) to determine the fixed effect structure. We tested the effects of *sex*, *temperature*, *extramolting* and every single interaction between pairs of factors on adult morphometrics and on larval coloration. We performed a backward stepwise model selection by Akaike Information Criterion and tested the significance of factors present in the selected model with a t-test. Briefly, *temperature* had a significant effect on the E/F ratio, on the green color and the brightness whereas *sex* had a significant effect on the geometric size (GM) and on F/C (Table S2). In addition, the interaction between *sex* and *temperature* had a significant effect on all ratios except H/P. *Extramolting* and the interaction between *sex* and *extramolting* had a significant effect on GM (Table S2). On this basis and even if all effects were not statistically significant for all traits, we fitted all three effects (sex, temperature and extramolting) and their interactions as fixed effects in all animal models for consistency between traits.

Table S2. Effects of extra-molting, sex and temperature on the 8 phase traits measured in the desert locust. For each trait, we show only variables with a significant effect. For %Green and Brightness, the results are presented for transformed variables (see Methods). No extramolt, male and 34°C were the reference levels of the tested parameters. *Brightness*: Level of brightness, which is inversely related to the level of black pigmentation; *%Green*: Percentage of

green color; *E*: Length of the fore wing; *F*: Length of the hind femur; *C*: Maximum width of the head; *H*: Height of the pronotum; *P*: Length of the pronotum; *O*: Vertical diameter of eyes; V: the width of the vertex between eyes.

Trait	Selected variables	Estimate	Std. Error	t	p-value
%Green	(Intercept)	0.454	0.004	112.809	< 2e-16
	Sex	-0.023	0.005	-4.143	0.000
1/Brightness	(Intercept)	0.009	0.000	37.393	<2e-16
	T°	-0.001	0.000	-4.500	0.000
E/F	(Intercept)	2.021	0.010	206.304	<2e-16
	T°	0.071	0.015	4.793	0.000
	Sex:T°	-0.050	0.019	-2.570	0.011
F/C	(Intercept)	3.946	0.029	136.694	<2e-16
	Sex	-0.176	0.039	-4.457	0.000
	Sex:T°	0.135	0.057	2.365	0.019
F/V	(Intercept)	13.791	0.237	58.174	<2e-16
	Sex:T°	1.069	0.469	2.279	0.024
O/V	(Intercept)	1.992	0.057	34.834	<2e-16
	Sex:T°	0.158	0.079	1.988	0.048
F	(Intercept)	3.436	0.015	222.291	< 2e-16
	Sex	-0.104	0.021	-4.938	0.000
	Sex:T°	0.091	0.031	2.980	0.003
Max larval weight	(Intercept)	1.886	0.039	48.088	< 2e-16
	ExtraMolt	0.387	0.040	9.651	< 2e-16
	Sex	-0.582	0.047	-12.435	< 2e-16
	ExtraMolt:Sex	-0.163	0.051	-3.159	0.002

1.5- Estimates of genetic parameters of phase variables at 28°C and 34°C

Table S3. Genetic parameters for morphological and colour traits of the desert locust estimated from with Model 1 including pedigree only as random effect, estimated on locusts reared either at 28°C or at 34°C. We presented values for phenotypic mean and variance (computed on raw data), additive genetic variance (V_A) and residual variance (V_R), heritability (h^2) and its standard error (SE), p-values of the pedigree effect. Sample sizes are 112 and 73 for larval

traits at 28°C and 34°, respectively, and for 108 and 104 adult traits at 28°C and 34°, respectively. Brightness: Level of brightness, which is inversely related to the level of black pigmentation; *Brightness*: Level of brightness, which is inversely related to the level of black pigmentation; *%Green*: Percentage of green color; *E*: Length of the fore wing; *F*: Length of the hind femur; *C*: Maximum width of the head; *H*: Height of the pronotum; *P*: Length of the pronotum; *O*: Vertical diameter of eyes ; V: the width of the vertex between eyes.

	28°C					34°C				
Trait	V_A	V_R	h^2	SE(h^2)	p-value (pedigree)	V_A	V_R	h^2	SE(h^2)	p-value (pedigree)
%Green	1.39E-03	9.59E-08	1.00	0.00	0.00	1.24E-10	1.22E-03	0.00	0.00	1.00
1/Brightness	5.12E-07	9.91E-07	0.34	0.23	0.02	3.07E-07	1.10E-06	0.22	0.27	0.29
EF	3.29E-09	4.81E-03	0.00	0.00	1.00	4.13E-04	4.42E-03	0.09	0.17	0.60
FC	1.93E-02	2.91E-02	0.40	0.25	0.04	2.43E-03	3.79E-02	0.06	0.14	0.61
FV	4.98E-01	2.64E+00	0.16	0.22	0.13	1.61E-06	2.56E+00	0.00	0.00	1.00
OV	1.13E-02	7.00E-02	0.14	0.15	0.14	9.44E-08	5.90E-02	0.00	0.00	1.00
F	4.36E-03	7.56E-03	0.37	0.23	0.02	2.17E-03	1.10E-02	0.17	0.18	0.18
Max larval weight	1.69E-02	1.05E-02	0.62	0.27	0.00	4.90E-03	2.84E-02	0.15	0.19	0.40

2- Details on methods and results from simulated data sets

2.1- Parameter values for crossing schemes

Table S4: Simulated crossing schemes. Crossing schemes are defined by D_S_O_N where D is the number of dams by sire, S the number of sires and O the number of offspring per dam and N the total sample size. F is the number of families (D x S) and D:S is the ratio of the number of dams on the number of sires. *: reference crossing scheme (mimicking our biological dataset); # derived from CS12.

CS	S	D	O	N	F	D:S
CS1	*8*	*2*	*13*	*208*	16	<1
CS2	4	2	26	208	8	<1
CS3*	13	2	8	208	26	<1
CS4	26	2	4	208	52	<1
CS5	13	4	4	208	52	<1
CS6	26	4	2	208	104	<1
CS7	13	8	2	208	104	<1
CS8	2	4	26	208	8	>1
CS9	2	8	13	208	16	>1
CS10	2	13	8	208	26	>1
CS11	4	13	4	208	52	>1
CS12	8	13	2	208	104	>1
CS13	2	26	4	208	52	>1
CS14	4	26	2	208	104	>1
CS15#	8	13	4	416	104	>1

2.2- Genetic parameters for one of the best simulated crossing scheme with 208 individuals (CS12)

Figure S3. Performance of heritability estimates evaluated from simulation datasets based on an optimized crossing scheme. We show mean estimate (h^2) and 95% confidence interval (empty circles and grey area, respectively), root mean square error (RMSE) (black squares) and percentage of simulations with significant pedigree effect (crosses) (y-axis) as a function of simulated h^2 (x-axis) and maternal effects (horizontal panels) . We used either Model 1 (without specified maternal effect, top panels) or Model 2 (specifying a maternal effect, bottom panels). Details on parameter values for the crossing scheme CS12 can be found in Table S4.

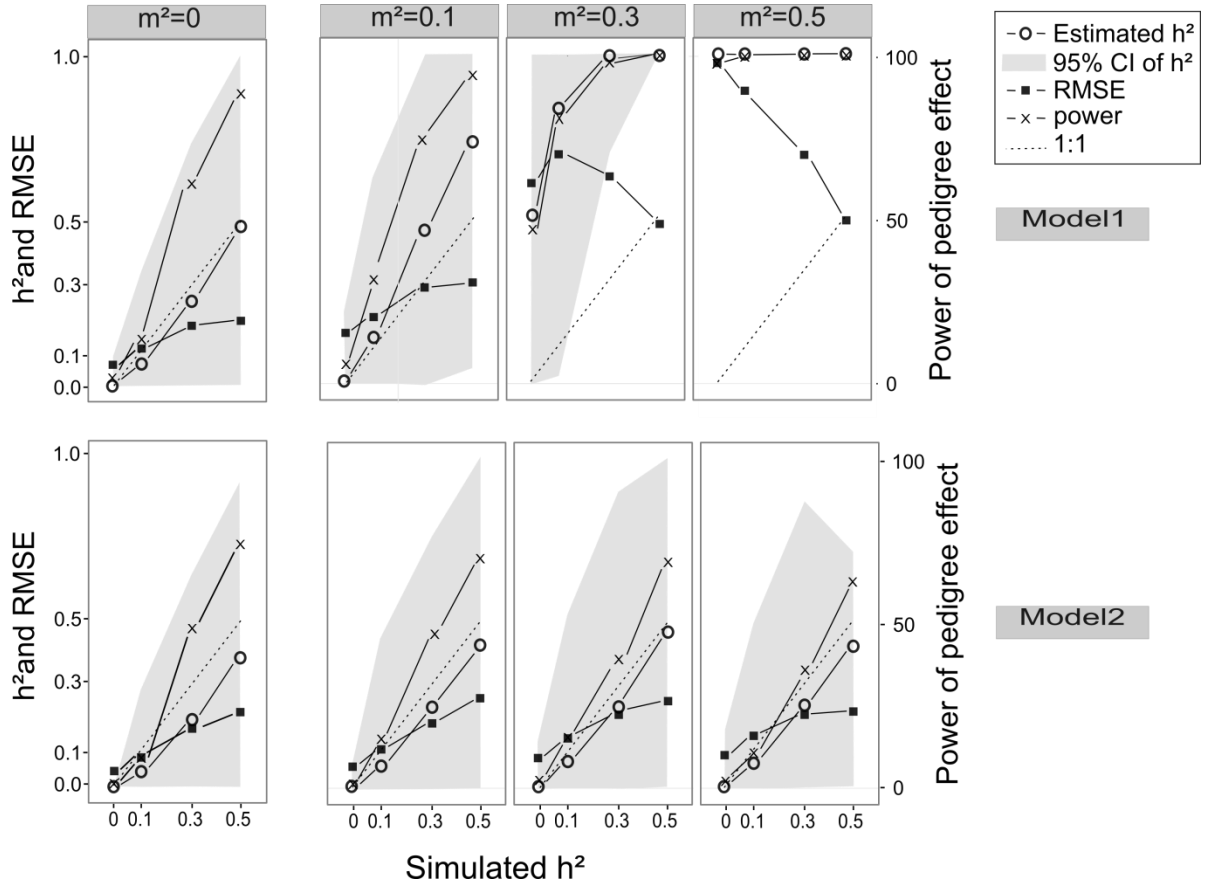
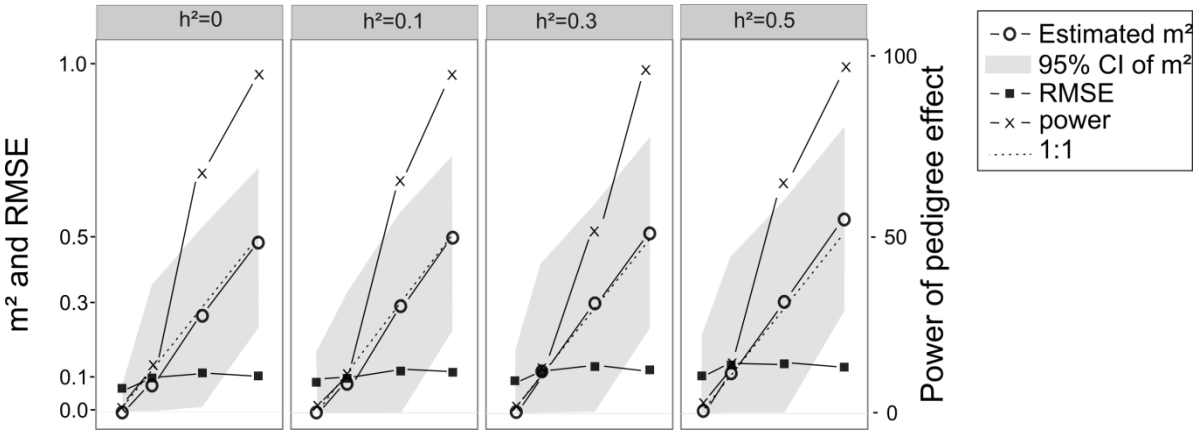


Figure S4. Performance of maternal effects estimation evaluated from simulation datasets based on an optimized crossing scheme. We show mean estimates (m^2) and 95% confidence intervals (empty circles and grey area, respectively), root mean square error (RMSE) (black squares) and percentage of simulations with significant maternal effect (crosses) as a function of simulated m^2 (x-axis) and simulated h^2 (panels). Estimates were obtained with Model 2. Details on parameter values for the crossing scheme CS12 can be found in Table S4.



3- References

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