SUPPLEMENTARY MATERIAL TO ARTICLE

Genetic relatedness does not predict the queen's successors in the primitively eusocial wasp, *Ropalidia marginata*

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Table S1: Descriptive statistics for the loci included in this study. The number of individuals (*n*), number of alleles per locus (*A*), the expected (H_e) and observed heterozygosity (H_o), and inbreeding coefficient (*f*) are shown.

	Queen succession
Rmsat11	
n	52
A	5
He	0.79
Ho	0.87
f	-0.10
Rmsat13	
n	60
A	5
He	0.66
Ho	0.77
f	-0.16
Rmsat83	
n	59
A	6
He	0.77
Ho	0.86
f	-0.12
Rmsat67	
n	60
A	2
He	0.14
Ho	0.15
f	-0.07
Rmsat74	
n	60
A	6
He	0.77
Ho	0.85
f	-0.10
Rmsat19	
n	60
Alleles	7
He	0.73

	Queen succession
Ho	0.90
f	-0.23
Rmsat65	
n	59
A	5
He	0.71
Ho	0.83
f	-0.17
All	
n	58.57
A	5.14
He	0.65
Ho	0.75
f	-0.15

Details of the models tested in this study

A) Definition

The rationale for the models can be broadly described as follows:

Model 1: The successors to the queen are decided based on the queen's inclusive fitness

Model 2: At any point in time the successor is decided based on the inclusive fitness of her immediate predecessor

Model 3: Each female selects the nestmate that would maximize her own inclusive fitness, and the one favoured by the most becomes the successor

Model 4: The colony members select the individual that would maximize the average inclusive fitness of the colony as their successor

The models are summarized in **Table S2**.

Table S2: Description of the models tested

Model 1	**Model 2**	**Model 3**	**Model 4**
The potential queens (PQs) at a particular point in time are decided based on the queen's relatedness to her nestmates. Test: A hierarchy is constructed by arranging the queen's relatedness with her nestmates in descending order and queue jumping analysis is done by comparing this with the hierarchy observed in serial PQ removal experiment.	The successor$_{n+1}$ at any point in time is decided based on the nestmate relatedness values of successor$_n$. The PQ1 is most related to the queen, PQ2 has the highest relatedness with PQ1, and so on. Test: A hierarchy is constructed for the queen and the PQs by arranging their relatedness with the rest of the nestmates in descending order. Queue jumping analysis is done for the queen (or respective the PQs) by comparing this hierarchy with the natural one.	Every individual votes for her own favourite individual who is decided by the individual with whom she is maximally related besides herself. The one who receives maximum votes becomes PQ1, the next highest become PQ2 etc. Test: A hierarchy is constructed by arranging the votes in descending order. And a queue jumping analysis is performed by comparing this hierarchy with the natural hierarchy observed in serial PQ removal experiments.	The individual that would maximize the average inclusive fitness of the colony becomes the successor. Test: R_i (definition in main text) is calculated for each individual and a hierarchy is constructed by arranging the R_i values in descending order. This hierarchy is compared with the natural hierarchy obtained from serial PQ removal experiment, by queue jumping analysis.

B) Description

Below, the models are illustrated for an imaginary colony consisting of four individuals, i.e., a, b, c and d, whose relatedness matrix can be given as

	a	b	c (Q)	d
a	r_{aa}	r_{ab}	r_{ac}	r_{ad}
b	r_{ba}	r_{bb}	r_{bc}	r_{bd}
c (Q)	r_{ca}	r_{cb}	r_{cc}	r_{cd}
d	r_{da}	r_{db}	r_{dc}	r_{dd}

Note that here, the above half of the matrix is a mirror image of the lower half and the diagonal $r_{aa,}$ $r_{bb,}$ r_{cc}etc., are all 1.

Model 1: According to this model the successor is that individual who has the highest relatedness to the queen. The model is illustrated below.

	a	b	c (Q)	d
a	r_{aa}	r_{ab}	r_{ac}	r_{ad}
b	r_{ba}	r_{bb}	r_{bc}	r_{bd}
c (Q)	r_{ca}	r_{cb}	r_{cc}	r_{cd}
d	r_{da}	r_{db}	r_{dc}	r_{dd}

a, b, c, d are nest mates. r_{ij} is the pair-wise genetic relatedness between the *i*th and the *j*th individual. Here individual c is the queen. I test the prediction that the individual with the highest

r_{cj} becomes PQ1, one with the second highest r_{cj} becomes PQ2 and so on. Accordingly a predicted hierarchy is constructed. The queen's own relatedness to herself is excluded from this analysis, as one is always related to oneself by 1 and hence would vote for herself.

The queen is automatically excluded in the queue jumping analysis (see Materials and Methods section).

Model 2: This is an extension of Model 1.i. According to this model the individual that is maximally related with successor$_n$ becomes successor$_{(n+1)}$, i.e., this model predicts that PQ1 has highest relatedness with queen, PQ2 with PQ1 and so on.

Model 3: According to this model, the favourite successor for each individual in the colony is the nestmate who is maximally related to her (except herself as relatedness to oneself is always 1). The nestmate who is favoured by the highest number of individuals becomes PQ1, the second highest becomes PQ2 and so on. A schematic description of this model is shown below.

	a	B	c (Q)	d
a	r_{aa}	r_{ab}	r_{ac}	r_{ad}
b	r_{ba}	r_{bb}	r_{bc}	r_{bd}
c (Q)	r_{ca}	r_{cb}	r_{cc}	r_{cd}
d	r_{da}	r_{db}	r_{dc}	r_{dd}

Here, I test the prediction that the individual *i* votes for the nestmate (excluding herself) with the highest r_{ij} value. And the one with the highest votes goes on to become the next successor. And a predicted hierarchy is constructed. In case of ties she can vote for more than one nestmate.

Model 4: To test this model, we define the following parameter for each individual:

$$R_i = \frac{\sum_{j=1}^{N} r_{ij}}{N}$$

Where r_{ij} is the relatedness of individual i with the jth individual. N is the size of the colony. I predict that the individual with the highest R_i becomes the PQ1, the second highest becomes PQ2 and so on, and a hierarchy is constructed based on that. The model is illustrated below.

	a	B	c (Q)	d	R
a	r_{aa}	r_{ab}	r_{ac}	r_{ad}	R_a
b	r_{ba}	r_{bb}	r_{bc}	r_{bd}	R_b
c (Q)	r_{ca}	r_{cb}	r_{cc}	r_{cd}	R_c
d	r_{da}	r_{db}	r_{dc}	r_{dd}	R_d

a, b, c, d are nest mates, R is the individual average pair-wise genetic relatedness. I test the prediction that the individual with the highest R_i goes on to become the successor.

C) Results

Table S3: Summary tables for the queue jumping analyses for each of the models tested in this study. The number at the heading for box refers to the model number in **Table S2**.

Model 1

Colony	**PQ1**	**PQ2**	**PQ3**	**PQ4**	**PQ5**	**Mean ± SD**
V1131	0.5	6	7	3.5	5	4.40 ± 2.53
V1146	1.5	13	5	8	1	5.70 ± 4.97
V1157	0	4	10.5	10	1.5	5.20 ± 4.83
V1158	6	5.5	5	4.5	4	5.00 ± 0.79
Mean±SD	2.0 ±2.74	7.13 ± 4.01	6.88 ± 2.59	6.50 ± 3.03	2.88 ± 1.93	
				Overall mean ± SD		**5.08 ± 3.44**

Model 2

Colony	**PQ1**	**PQ2**	**PQ3**	**PQ4**	**PQ5**	**Mean±SD**
V1131	0.5	1	7	3.5	3	3.00 ± 2.57
V1146	1.5	13	6	9	8	7.50 ± 4.21
V1157	0	14	7	2	10	6.60 ± 5.73
V1158	6	9	1	5	5.5	5.30 ± 2.86
Mean±SD	2.00 ± 2.74	9.25 ± 5.91	5.25 ± 2.87	4.88 ± 3.01	6.63 ± 3.04	
				Overall mean ± SD		**5.60 ± 4.10**

Model 3

Colony	**PQ1**	**PQ2**	**PQ3**	**PQ4**	**PQ5**	**Mean±SD**
V1131	2.5	0	8.5	3.5	2.5	3.40 ± 3.88
V1146	6	11.5	5.5	6.5	5	6.90 ± 2.63
V1157	6.5	6	11	0	4.5	5.60 ± 3.96
V1158	11.5	11	7	6.5	1.5	7.50 ± 4.05
Mean±SD	6.63 ± 3.71	7.13 ± 5.36	8.00 ± 2.35	4.13 ± 3.09	3.38 ± 1.65	
				Overall mean ± SD		**5.85 ± 3.59**

Model 4						
Colony	**PQ1**	**PQ2**	**PQ3**	**PQ4**	**PQ5**	**Mean±SD**
V1131	1.5	0	8	2	2	2.70 ± 3.07
V1146	6	13	5.5	8	5	7.50 ± 3.28
V1157	14	5	8	0	9	7.20 ± 5.17
V1158	7	9	5	5	1.5	5.50 ± 2.78
Mean±SD	7.13 ± 5.17	6.75 ± 5.56	6.63 ± 1.60	3.75 ± 1.63	4.38 ± 3.45	
				Overall mean ± SD		**5.73 ± 3.92**

D) Distribution of relatedness (r) in the four colonies

Colony ID: 1131

Individual ID	XX(PQ3)	OO(PQ2)	_Y	_B	LL	B_	YY	R_ (Q)	SS(PQ5)	_R(PQ1)	_O(PQ4)	O_
XX(PQ3)		0.5	0.5	0.5	0.5	0.5	0.5	0.08	0.5	0.5	0.5	0.5
OO(PQ2)	0.5		0.42	0.56	0.66	0.88	0.81	0.2	0.73	0.77	0.61	0.77
_Y	0.5	0.42		0.67	0.53	0.37	0.65	0.24	0.5	0.54	0.87	0.54
_B	0.5	0.56	0.67		0.46	0.68	0.65	0.29	0.73	0.59	0.69	0.59
LL	0.5	0.66	0.53	0.46		0.78	0.51	0.26	0.72	0.5	0.71	0.5
B_	0.5	0.88	0.37	0.68	0.78		0.69	0.25	0.73	0.65	0.5	0.65
YY	0.5	0.81	0.65	0.65	0.51	0.69		0	0.6	0.65	0.61	0.65
R_ (Q)	0.08	0.2	0.24	0.29	0.26	0.25	0		0.11	0.37	0.25	0.37
SS(PQ5)	0.5	0.73	0.5	0.73	0.72	0.73	0.6	0.11		0.6	0.65	0.6
_R(PQ1)	0.5	0.77	0.54	0.59	0.5	0.65	0.65	0.37	0.6		0.63	1
_O(PQ4)	0.5	0.61	0.87	0.69	0.71	0.5	0.61	0.25	0.65	0.63		0.63
O_	0.5	0.77	0.54	0.59	0.5	0.65	0.65	0.37	0.6	1	0.63	

Mean: 0.55 ± 0.19

Colony ID: V1146

Individual ID	_Y	RS	SS(PQ2)	RY(Q)	RR(PQ1)	RB	_S	_R(PQ4)	L_	XX	RD	DD(PQ5)	X_	R_	RL	OO(PQ3)
_Y		0.8	0	0.3	0.6	0.35	1	0.54	0.59	0.81	0.82	0.6	0.44	0.67	0.51	0.79
RS	0.8		0	0.53	0.8	0.62	0.8	0.77	0.8	0.6	0.62	0.8	0.65	0.5	0.67	0.59
SS(PQ2)	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
RY(Q)	0.3	0.53	0		0.77	0.66	0.3	0.39	0.79	0.56	0.13	0.77	0.66	0.55	0.67	0.65
RR(PQ1)	0.6	0.8	0	0.77		0.76	0.6	0.52	0.58	0.8	0.42	1	0.37	0.32	0.49	0.3
RB	0.35	0.62	0	0.66	0.76		0.35	0.77	0.39	0.64	0.57	0.76	0.67	0.03	0.57	0.33
_S	1	0.8	0	0.3	0.6	0.35		0.54	0.59	0.81	0.82	0.6	0.44	0.67	0.51	0.79
_R(PQ4)	0.54	0.77	0	0.39	0.52	0.77	0.54		0.61	0.28	0.77	0.52	0.81	0.25	0.81	0.41
L_	0.59	0.8	0	0.79	0.58	0.39	0.59	0.61		0.36	0.41	0.58	0.8	0.74	0.8	0.8
XX	0.81	0.6	0	0.56	0.8	0.64	0.81	0.28	0.36		0.63	0.8	0.13	0.5	0.33	0.56
RD	0.82	0.62	0	0.13	0.42	0.57	0.82	0.77	0.41	0.63		0.42	0.61	0.53	0.67	0.67
DD(PQ5)	0.6	0.8	0	0.77	1	0.76	0.6	0.52	0.58	0.8	0.42		0.37	0.32	0.49	0.3
X_	0.44	0.65	0	0.66	0.37	0.67	0.44	0.81	0.8	0.13	0.61	0.37		0.58	0.9	0.67
R_	0.67	0.5	0	0.55	0.32	0.03	0.67	0.25	0.74	0.5	0.53	0.32	0.58		0.63	0.79
RL	0.51	0.67	0	0.67	0.49	0.57	0.51	0.81	0.8	0.33	0.67	0.49	0.9	0.63		0.68

OO(PQ3)	0.79	0.59	0	0.65	0.3	0.33	0.79	0.41	0.8	0.56	0.67	0.3	0.67	0.79	0.68

Mean: 0.52 ± 0.27

Colony ID: V1157

Individual ID	W_	_W	XX(PQ1)	_B(PQ3)	RY	SS(PQ2)	_R(PQ4)	_O(PQ5)	OO(Q)	YY	B_	_X	_L	X_	Y_	_D	O_
W_		0.72	0.47	0.56	0	0.62	0.88	0.52	0	0.55	0.56	0.43	0.51	0.65	0.77	0.76	0.53
_W	0.72		0.17	0.56	0	0.69	0.54	0.72	0.5	0.11	0.5	0.31	0.15	0.23	0.5	0.55	0.36
XX(PQ1)	0.47	0.17		0.51	0.5	0	0.47	0.5	0.62	0.51	0.3	0.18	0.5	0.07	0.51	0.5	0.5
_B(PQ3)	0.56	0.56	0.51		0	0.6	0.73	0.22	0	0.71	0.61	0.56	0.58	0.5	0.86	0.87	0.61
RY	0	0	0.5	0		0	0	0.5	0.57	0.03	0	0	0.09	0	0	0	0.43
SS(PQ2)	0.62	0.69	0	0.6	0		0.81	0.5	0.26	0.59	0.78	0.76	0.56	0.66	0.71	0.77	0.52
_R(PQ4)	0.88	0.54	0.47	0.73	0	0.81		0.34	0	0.73	0.68	0.66	0.67	0.77	0.89	0.88	0.64
_O(PQ5)	0.52	0.72	0.5	0.22	0.5	0.5	0.34		0.5	0.5	0.49	0.35	0.55	0.47	0.23	0.45	0.78
OO(Q)	0	0.5	0.62	0	0.57	0.26	0	0.5		0.21	0.05	0.04	0.22	0	0	0	0.43
YY	0.55	0.11	0.51	0.71	0.03	0.59	0.73	0.5	0.21		0.6	0.72	0.87	0.6	0.86	0.56	0.64
B_	0.56	0.5	0.3	0.61	0	0.78	0.68	0.49	0.05	0.6		0.77	0.78	0.67	0.78	0.57	0.55
_X	0.43	0.31	0.18	0.56	0	0.76	0.66	0.35	0.04	0.72	0.77		0.67	0.67	0.77	0.53	0.5
_L	0.51	0.15	0.5	0.58	0.09	0.56	0.67	0.55	0.22	0.87	0.78	0.67		0.78	0.77	0.54	0.79
X_	0.65	0.23	0.07	0.5	0	0.66	0.77	0.47	0	0.6	0.67	0.67	0.78		0.67	0.64	0.77

Y_	0.77	0.5	0.51	0.86	0	0.71	0.89	0.23	0	0.86	0.78	0.77	0.77	0.67		0.77	0.54
_D	0.76	0.55	0.5	0.87	0	0.77	0.88	0.45	0	0.56	0.57	0.53	0.54	0.64	0.77		0.77
O_	0.53	0.36	0.5	0.61	0.43	0.52	0.64	0.78	0.43	0.64	0.55	0.5	0.79	0.77	0.54	0.77	

Mean: 0.49 ± 0.26

Colony ID: V1158

Individual ID	_S	DD	XX(PQ5)	OO	_Y	_O(PQ1)	X_	WO(W_)	B_(PQ2)	_D	Y_(Q)	RR	YY(PQ4)	SS(PQ3)	D_
_S		0.63	0.63	0.5	0	0.74	0.45	0.5	0.18	0.78	0.5	0.27	0.67	0.52	0.67
DD	0.63		1	0.66	0	0.71	0.38	0.79	0.58	0.67	0.5	0.63	0.57	0.67	0.79
XX(PQ5)	0.63	1		0.66	0	0.71	0.38	0.79	0.58	0.67	0.5	0.63	0.57	0.67	0.79
OO	0.5	0.66	0.66		0.13	0.68	0.34	0.64	0.78	0.64	0.5	0.81	0.76	0.64	0.4
_Y	0	0	0	0.13		0.06	0	0	0.13	0	0.32	0	0.2	0.1	0

_O(PQ1)	0.74	0.71	0.71	0.68	0.06		0.39	0.71	0.5	0.68	0.5	0.56	0.5	0.68	0.5
X_	0.45	0.38	0.38	0.34	0	0.39		0.73	0.45	0.79	0.5	0.34	0.57	0.57	0.76
WO(W_)	0.5	0.79	0.79	0.64	0	0.71	0.73		0.64	0.67	0.5	0.63	0.57	0.67	0.79
B_(PQ2)	0.18	0.58	0.58	0.78	0.13	0.5	0.45	0.64		0.34	0.5	0.46	0.53	0.69	0.48
_D	0.78	0.67	0.67	0.64	0	0.68	0.79	0.67	0.34		0.5	0.49	0.82	0.82	0.7
Y_(Q)	0.5	0.5	0.5	0.5	0.32	0.5	0.5	0.5	0.5	0.5		0.07	0.5	0.5	0.54
RR	0.27	0.63	0.63	0.81	0	0.56	0.34	0.63	0.46	0.49	0.07		0.49	0.5	0.31
YY(PQ4)	0.67	0.57	0.57	0.76	0.2	0.5	0.57	0.57	0.53	0.82	0.5	0.49		0.63	0.61
SS(PQ3)	0.52	0.67	0.67	0.64	0.1	0.68	0.57	0.67	0.69	0.82	0.5	0.5	0.63		0.41
D_	0.67	0.79	0.79	0.4	0	0.5	0.76	0.79	0.48	0.7	0.54	0.31	0.61	0.41	

Mean: 0.52 ± 0.23