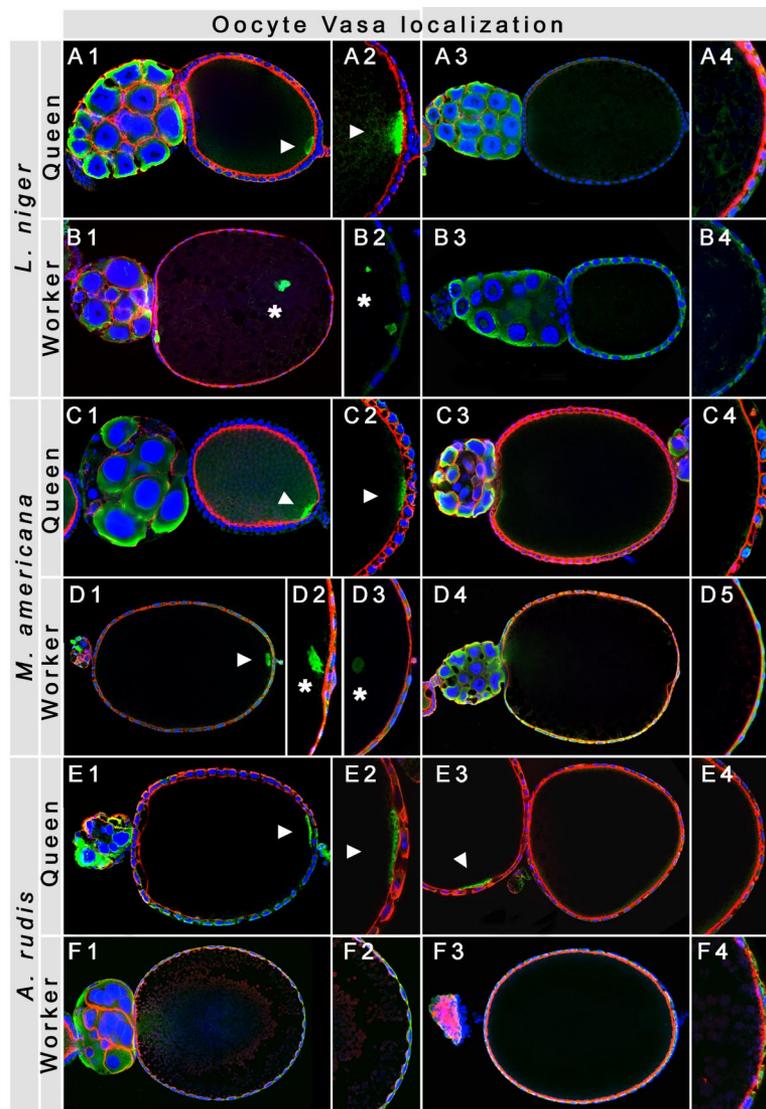


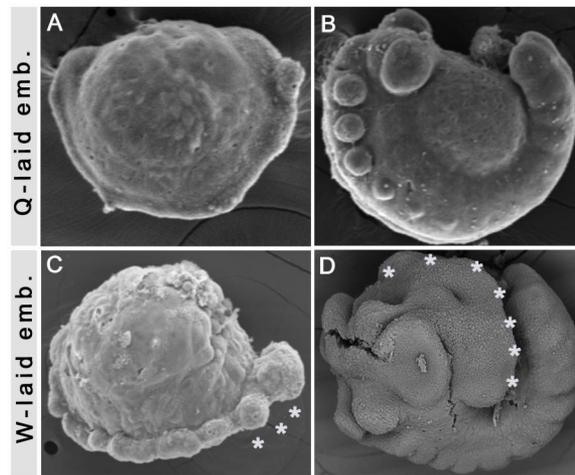
# Supporting Information

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**Fig. S1.** Patterns of Vasa protein localization in the oocytes of *A. rudis*, *M. americana* and *L. niger* queens and workers. Green color marks Vasa protein; red marks F-actin and blue marks Nuclei. Arrow heads indicate correct Vasa localization; asterisks indicate impaired Vasa localization. In the queens of *L. niger* (A), *M. americana* (C), and *A. rudis* (E), posterior localization Vasa (A1–2, C1–2, and E1–2) indicates viable oocytes, whereas the lack Vasa localization (A3–4; C3–4, and E3–4) indicates trophic oocytes. In the workers of *L. niger* (B), *M. americana* (D), and *A. rudis* (F), correct Vasa localization (D1) indicates viable oocytes; impaired Vasa localization (B1–2; D2–3) indicates failed oocytes, while absence of Vasa localization (B3–4, D4–5, and F1–4) indicates trophic oocytes.





**Fig. S3.** Morphological assessment of embryonic development in *A. rudis* queen- and worker-laid eggs. Asterisks indicate embryonic defects. (*A* and *B*) Embryos produced by the queen showing normal development during gastrulation (*A*) and late segmentation (*B*). (*C* and *D*) Embryos produced by the workers, showing axis defects during gastrulation (*C*) and segmentation (*D*).

Table S1. Pattern and frequency of oocyte production in *A. rudis*, *M. americana*, and *L. niger*.

	Viable (Vasa <sup>+</sup> )	Trophic (Vasa <sup>-</sup> )	Failed (Vasa*)
<i>A. rudis</i>			
Queen <sup>†</sup>	62	34	0
Queenright worker			
Replicate 1	0	101	0
Replicate 2	0	115	0
Replicate 3	0	29	0
Orphaned worker			
Replicate 1	46	76	0
Replicate 2	22	59	6
Replicate 3	4	41	3
<i>M. americana</i>			
Queen <sup>†</sup>	34	7	0
Queenright worker			
Replicate 1	4	0	19
Replicate 2	1	0	4
Replicate 3	7	2	41
Orphaned worker			
Replicate 1	9	2	32
Replicate 2	9	3	28
Replicate 3	6	0	14
<i>L. niger</i>			
Queen <sup>†</sup>	29	19	0
Queenright worker			
Replicate 1	0	27	8
Replicate 2	0	20	4
Replicate 3	1	15	7
Orphaned worker			
Replicate 1	0	0	13
Replicate 2	0	25	11
Replicate 3	1	8	11

<sup>†</sup>The percentage of trophic eggs in queens may vary according to their age. Young queens are known to produce higher numbers of trophic eggs relative to mature queens.

Table S2. Embryo production and development in *A. rudis* and *M. americana* workers

	Normal	Trophic*	Arrested
<i>A. rudis</i>			
Queen-eggs			
Replicate 1	53	5	0
Replicate 2	12	0	0
Replicate 3	18	0	0
Orphaned worker-eggs			
Replicate 1	80	5	3
Replicate 2	37	4	0
Replicate 3	59	10	7
<i>M. americana</i>			
Queen-eggs			
Replicate1	96	0	0
Replicate2	62	4	0
Replicate3	26	2	0
Orphaned worker-eggs			
Replicate 1	1	0	62
Replicate 2	5	0	30
Replicate 3	3	0	38

\*Trophic eggs are eaten as they are produced, which may explain the variation between the numbers of trophic oocytes produced (Table S1) and the numbers of trophic eggs found.

**Table S3. Reproductive constraint assessment based on the percentage of failed oocytes in orphaned workers**

	Viable (Vasa <sup>+</sup> )	Trophic (Vasa <sup>-</sup> )	Failed (Vasa*)	Degree of reproductive constraint, % <sup>†</sup>
<i>Camponotus floridanus</i>	26	2	23	47
<i>Camponotus sansebeanus</i>	0	1	23	100
<i>Formica dulosa</i>	25	14	57	70
<i>Formica subsericia</i>	3	6	12	80
<i>Lasius niger</i> <sup>‡</sup>	1	33	35	97
<i>Lasius alienus</i>	9	–	2	18
<i>Myrmica americana</i> <sup>‡</sup>	24	5	74	76
<i>Aphenogaster rudis</i> <sup>‡</sup>	72	176	9	11
<i>Aphaenogaster treate</i>	14	8	2	13
<i>Leptothorax regulatus</i>	5	10	10	67

<sup>†</sup>The degree of reproductive constraint (Fig. 4) was calculated based on the percentage of failed oocytes relative to viable oocytes. Trophic oocytes are not produced for reproduction purposes and were therefore excluded.

<sup>‡</sup>Species analyzed in detail.