Supporting Information

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SI Text

Survival Analysis. To examine whether adult mortality was affected by individual affiliation (worker/cheater, coded as 0/1), initial cheater proportion, or source colony ID, we applied Cox's proportional hazard model to the adult survival data throughout the experiment (Fig. S1). The source colony ID was treated as a fixed effect. The analysis was conducted using the "survival" package implemented in v3.0.0 of the R software (www.r-project.org). The mortality of cheaters was significantly lower than that of workers [slope ± SEM = –1.442 ± 0.081; likelihood-ratio test (LRT), 1 df, χ^2 = 313.6, P < 0.0001], and adult mortality increased significantly as the initial cheater proportion increased (slope ± SEM = 3.197 ± 0.133; LRT, 1 df, χ^2 = 614.9, P < 0.0001), and varied significantly by source colony ID (LRT, 4 df, χ^2 = 290.5, P < 0.0001; possibly reflecting initial conditions of the source colonies including genetic background). These results corroborate the analysis of fitness described in the main text.

Developmental Stage of the Brood. On days 16, 32, and 48 from the onset, we checked inside each nest for the presence and the developmental stage of the brood. We categorized the brood development into stage 0 (none), stage 1 (eggs only), or stage 2 (eggs and larvae). We treated the stage as an ordinal scale (stage 0 < stage 1 < stage 2), and used an ordinal logistic regression model to examine whether the developmental stage was explained by the initial proportion of cheaters in the colony, days from the onset, or source colony ID. The analysis was conducted using the "MASS" package implemented in the R software. Although most (23 of 25) of the experimental colonies produced eggs on day 16, those with 100% cheaters could not maintain them by day 32 (Fig. S2*A*). Consequently, the progression of brood development showed a significant negative association with the initial cheater proportion (slope ± SEM = –6.309 ± 1.320; LRT, 1 df, χ^2 = 40.90, P < 0.0001), which supports the progress of the public goods dilemma in the colonies. In addition, the brood development varied significantly by source colony ID (LRT, 4 df, χ^2 = 20.79, P < 0.0001), and showed positive (but not significant) association with days from the onset (slope ± SEM = 0.038 ± 0.023; LRT, 1 df, χ^2 = 3.026, P = 0.0819).

Hygiene Status of the Nest. As we checked the brood development, we also monitored the hygiene status of the nests. Relying on the quantity of waste materials on the floor of the nest (0–2 cm from the cotton plug), including dead adults and dead brood, we categorized the nest hygiene into level 0 (waste materials covered more than half of the floor), level 1 (wastes covered less than half of the floor), or level 2 (virtually no wastes inside the nest). We treated the level as an ordinal scale (level 0 < level 1 < level 2), and used an ordinal logistic regression model to examine whether the nest hygiene was explained by the initial proportion of cheaters in the colony, days from the onset, or source colony ID. The analysis was conducted using the "MASS" package implemented in the R software. The colonies with 100% cheaters showed deteriorated hygiene as early as day 16 (Fig. S2*B*; see also Fig. 1*D*). The nest hygiene showed a significant negative association with the initial cheater proportion (slope ± SEM = –9.381 ± 1.835; LRT, 1 df, χ^2 = 65.81, P < 0.0001), which indicates that increasing the proportion of cheaters caused a shortage of the inside-nest workforce. In addition, the nest hygiene got worse with time (effect of days from the onset; slope ± SEM = –0.087 ± 0.028; LRT, 1 df, χ^2 = 11.67, P = 0.0006), and varied significantly with source colony ID (LRT, 4 df, χ^2 = 12.90, P = 0.0118).

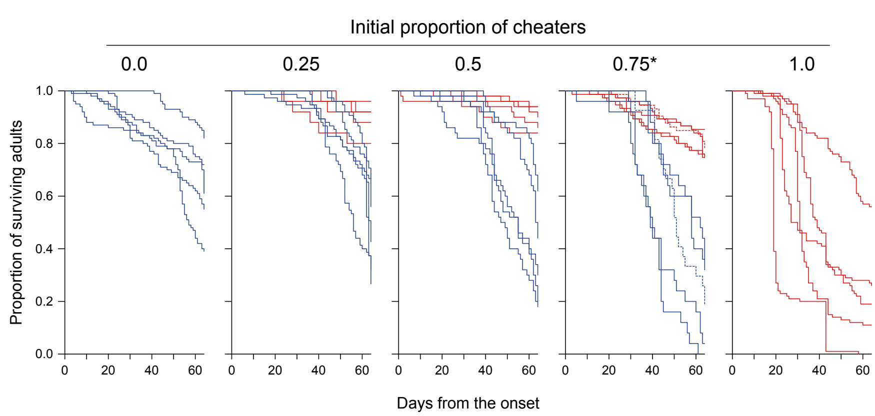

Fig. S1. Impact of initial cheater proportion on survival of adults. Lines indicate the survival of adults in a colony: red, cheaters; blue, workers. The adult mortality was significantly greater for workers than for cheaters, and increased significantly with the increase in the initial cheater proportion, as revealed by the survival analysis using Cox's proportional hazard model. *Dashed lines indicate the survival data of the colony with an initial cheater proportion of 0.73.

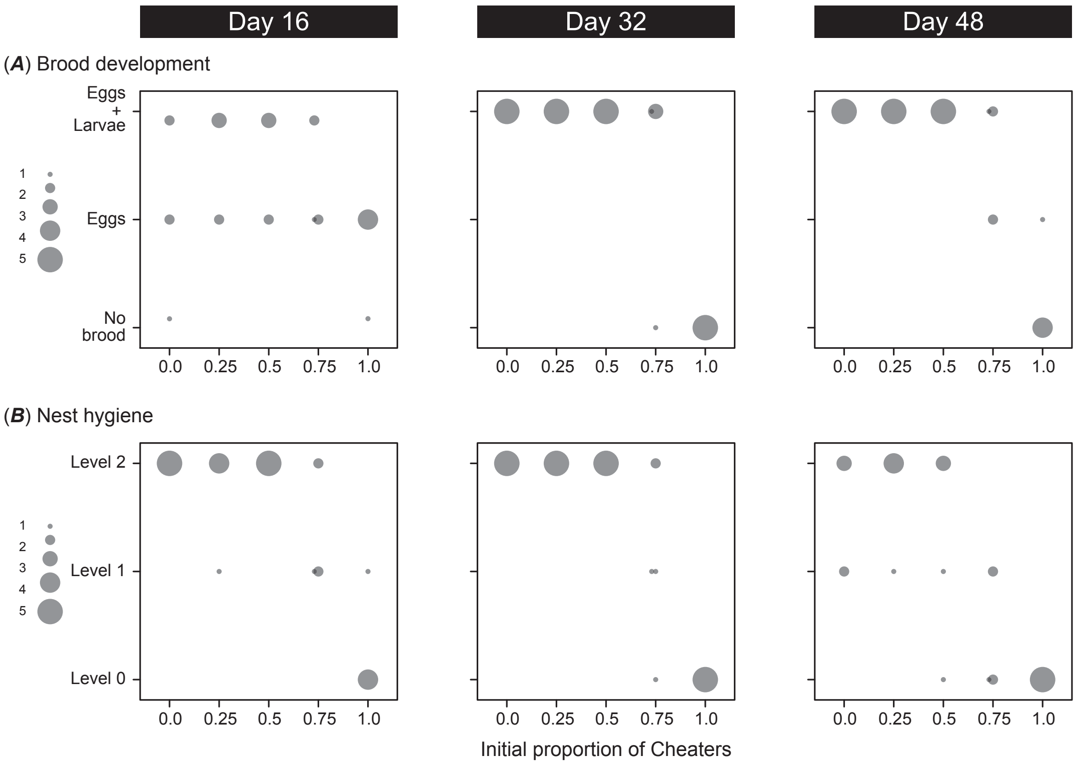

Fig. S2. Successive observations of (*A*) brood development and (*B*) nest hygiene status of colonies on days 16, 32, and 48. Circle size reflects sample size (number of colonies). See *SI Text* for details.

Table S1. Summary of the generalized linear mixed model (GLMM; with binomial errors and logit-link) fitted to the proportion of outside-nest workers

Explanatory variables	Coefficient	SEM	df	χ^2	*P*
(Intercept)	−3.782	0.166			
Initial cheater proportion	1.685	0.349	1	15.52	<0.0001
Days from the onset	0.054	0.001	1	1,526	<0.0001

The initial cheater proportion and days from the onset were treated as fixed explanatory variables, and experimental colony ID nested within source colony ID as a random intercept. The LRT showed that both explanatory variables were significantly associated with the outside-nest worker activity. The relative outside-nest activity of workers was computed as residuals from this fitting (see *Materials and Methods* for details).