Fig. S1. Antibacterial activity in spermathecae and control tissues of mated *Lasius niger* queens with corresponding egg laying rates.

The mean antibacterial activity (ABA) values (± SD) in the mated queen spermathecae (black dots) and control tissues (i.e. head-thorax homogenates; white squares) during the first 30 days after mating are given in the lower part of the figure. They reflect ABA relative to that of the negative control (i.e., free growth; see Methods). Values > 0 mean ABA was present; values = 0 mean there was no difference in activity between the sample type and the negative control; and values < 0 indicate that bacterial growth was enhanced. Statistically significant differences in ABA between consecutive time points are indicated in spermathecal contents and control tissues by capital and lower-case letters, respectively.

Mated queen egg laying rate over the first 30 days after mating is shown in the upper part of the figure. Horizontal bars represent the mean number of eggs laid per day (± SD) over the following periods of time: 0-1 day, 5-10 days, 10-20 and 20-30 days after mating. Given that mated queen egg laying rate was calculated as part of another study, these queens are not the same as the ones used for ABA measures. Sample sizes are indicated above (egg laying) or beneath (ABA) error bars.

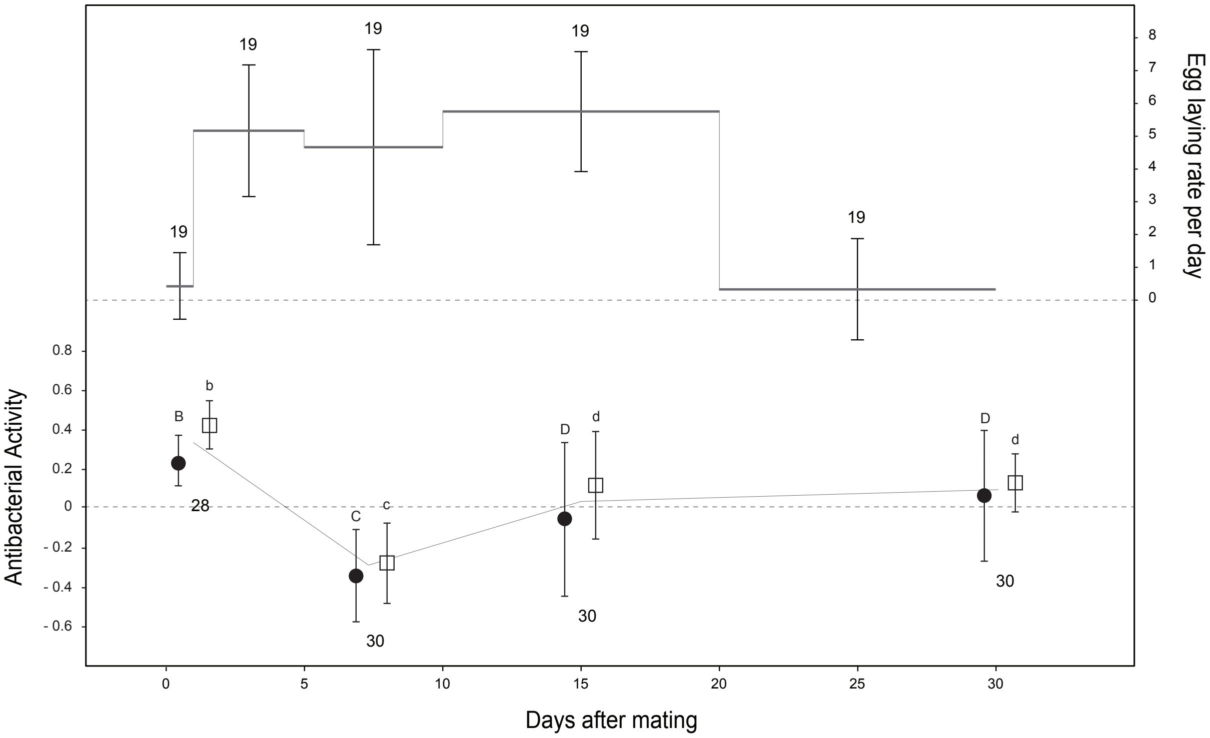

Table S1. Antibacterial activity (ABA) index of sperm-storage organ contents from males (accessory testes) and females (spermatheca of virgin queens (i.e., gynes), and of 1-day, 1-week, 2-week and 4-week mated queens), and of head-thorax homogenates (homo), in the ant *Lasius niger*.

The ABA index is calculated as ABA = 1 - (sample OD/negative control OD) (see Methods).

ABA	Tissue	Individual
-0.484793585	sperm	male
-0.071197236	sperm	male
-0.588625304	sperm	male
-0.142148911	sperm	male
-0.175028955	sperm	male
-0.342890235	sperm	male
-0.325584948	sperm	male
-0.555745259	sperm	male
-0.211370057	sperm	male
-0.124843625	sperm	male
-0.493446228	sperm	male
-0.453644069	sperm	male
-0.439799839	sperm	male
-0.114460453	sperm	male
-0.931269977	sperm	male
-0.150801554	sperm	male
-0.102346752	sperm	male
0.02398184	sperm	male
-0.277130146	sperm	male
-0.265016445	sperm	male
-0.348075188	sperm	male
-0.437137213	sperm	male
-0.639550905	sperm	male
-0.58557392	sperm	male
-0.542392333	sperm	male
-0.644948603	sperm	male
-0.107877607	sperm	male
-0.558585428	sperm	male
-0.653045151	sperm	male
-0.331882093	sperm	male
-0.561284277	sperm	male
-0.730997014	sperm	male
-0.334987893	sperm	male
-0.280986649	sperm	male
-0.242414332	sperm	male
-0.766997843	sperm	male
-0.378703185	sperm	male
-0.05726721	sperm	male
-0.622994526	sperm	male
-0.1957953	homo	male
-0.310010191	homo	male
-0.306549133	homo	male
0.151211918	homo	male
-0.79608416	homo	male
-0.751568916	homo	male
-0.345273844	homo	male

0.15268962	homo	male
0.498478626	homo	male
0.222458535	homo	male
0.236717876	homo	male
0.262180984	homo	male
0.05847612	homo	male
0.242829022	homo	male
0.539219598	homo	male
0.083939228	homo	male
-0.094302528	homo	male
0.562645658	homo	male
0.059494644	homo	male
-0.024449566	control-	male
-0.023194831	control-	male
0.011175651	control-	male
0.006032675	control-	male
0.030436071	control-	male
0.295103764	sperm	gyne
0.446152957	sperm	gyne
0.784869331	sperm	gyne
0.217290543	sperm	gyne
0.052509605	sperm	gyne
0.556006916	sperm	gyne
0.298808645	sperm	gyne
0.162363564	sperm	gyne
0.103116837	sperm	gyne
0.281372019	sperm	gyne
0.299730943	sperm	gyne
0.766560337	sperm	gyne
0.853528055	sperm	gyne
0.873210222	sperm	gyne
0.793108377	sperm	gyne
0.815079169	sperm	gyne
0.772968485	sperm	gyne
0.804551498	sperm	gyne
0.034200611	homo	gyne
0.272217522	homo	gyne
0.372916985	homo	gyne
-0.043612609	homo	gyne
-0.01614912	homo	gyne
0.272217522	homo	gyne
0.01131437	homo	gyne
0.061664101	homo	gyne
0.276794771	homo	gyne
-0.024449566	control-	gyne
-0.023194831	control-	gyne
0.011175651	control-	gyne
0.006032675	control-	gyne
0.030436071	control-	gyne
0.030049563	sperm	q1d
0.11728664	sperm	q1d
0.395008864	sperm	q1d
0.157706307	sperm	q1d
0.316777252	sperm	q1d
0.082203971	sperm	q1d
0.106855759	sperm	q1d

0.099154154	sperm	q1d
0.065132232	sperm	q1d
0.320688833	sperm	q1d
0.391097284	sperm	q1d
0.159010167	sperm	q1d
0.17987193	sperm	q1d
0.252888102	sperm	q1d
0.232026339	sperm	q1d
0.091209436	sperm	q1d
0.301130929	sperm	q1d
0.009066243	sperm	q1d
0.202037554	sperm	q1d
0.269838285	sperm	q1d
0.342854456	sperm	q1d
0.469328896	sperm	q1d
0.106855759	sperm	q1d
0.424997649	sperm	q1d
0.321992693	sperm	q1d
0.318081112	sperm	q1d
0.344158316	sperm	q1d
0.336335155	sperm	q1d
0.288092327	sperm	q1d
0.411959047	sperm	q1d
0.594499476	homo	q1d
0.271142145	homo	q1d
0.415870628	homo	q1d
0.55929525	homo	q1d
0.478455918	homo	q1d
0.554079809	homo	q1d
0.265926704	homo	q1d
0.483671358	homo	q1d
0.487582939	homo	q1d
0.580157014	homo	q1d
0.581460874	homo	q1d
0.541041207	homo	q1d
0.413262907	homo	q1d
0.530610326	homo	q1d
0.314169532	homo	q1d
0.316777252	homo	q1d
0.372843241	homo	q1d
0.554079809	homo	q1d
0.454986434	homo	q1d
0.477152057	homo	q1d
0.461505735	homo	q1d
0.385881843	homo	q1d
0.314169532	homo	q1d
0.298523209	homo	q1d
0.067739953	homo	q1d
0.418478348	homo	q1d
0.452378713	homo	q1d
0.310257951	homo	q1d
0.301130929	homo	q1d
0.358500779	homo	q1d
-0.052391843	control-	q1d
0.007803625	control-	q1d
0.017899075	control-	q1d

0.026689143	control-	q1d
-0.122775046	sperm	q1w
-0.475067893	sperm	q1w
-0.736119427	sperm	q1w
-0.231757725	sperm	q1w
-0.307792153	sperm	q1w
-0.705705656	sperm	q1w
-0.206412916	sperm	q1w
-0.18867155	sperm	q1w
-0.315395595	sperm	q1w
-0.401567946	sperm	q1w
-0.538429916	sperm	q1w
-0.649947076	sperm	q1w
-0.469998931	sperm	q1w
-0.122775046	sperm	q1w
0.014086924	sperm	q1w
-0.63220571	sperm	q1w
-0.072085427	sperm	q1w
-0.376223137	sperm	q1w
-0.335671443	sperm	q1w
0.03182829	sperm	q1w
-0.120240565	sperm	q1w
-0.414240351	sperm	q1w
-0.307792153	sperm	q1w
-0.16332674	sperm	q1w
-0.231757725	sperm	q1w
-0.693033252	sperm	q1w
-0.214016359	sperm	q1w
-0.82736074	sperm	q1w
-0.302723191	homo	q1w
-0.533360954	homo	q1w
-0.419309313	homo	q1w
-0.196274992	homo	q1w
-0.297654229	homo	q1w
-0.462395488	homo	q1w
-0.02393029	homo	q1w
-0.269774939	homo	q1w
-0.173464664	homo	q1w
-0.368619695	homo	q1w
-0.312861114	homo	q1w
-0.049275099	homo	q1w
-0.791878008	homo	q1w
-0.310326634	homo	q1w
-0.267240458	homo	q1w
-0.363550733	homo	q1w
-0.672757404	homo	q1w
-0.267240458	homo	q1w
-0.018861328	homo	q1w
-0.406636908	homo	q1w
-0.198809473	homo	q1w
-0.330602481	homo	q1w
-0.016326847	homo	q1w
0.039431733	homo	q1w
-0.026464771	homo	q1w
-0.092361275	homo	q1w
-0.525757511	homo	q1w

-0.089826794	homo	q1w
-0.46746445	homo	q1w
0.041341495	control-	q1w
-0.038241206	control-	q1w
-0.025568802	control-	q1w
-0.045303257	control-	q1w
0.013201583	control-	q1w
0.023013496	control-	q1w
0.03155669	control-	q1w
0.36060628	sperm	q2w
0.560562134	sperm	q2w
-0.136958288	sperm	q2w
0.869796188	sperm	q2w
0.035096749	sperm	q2w
0.049047158	sperm	q2w
0.093223451	sperm	q2w
-0.01372968	sperm	q2w
-0.506644112	sperm	q2w
-0.857729391	sperm	q2w
-0.615922312	sperm	q2w
0.26295342	sperm	q2w
0.009521	sperm	q2w
0.342005735	sperm	q2w
0.221102195	sperm	q2w
0.002545796	sperm	q2w
0.23970274	sperm	q2w
-0.399690981	sperm	q2w
-0.036980361	sperm	q2w
-0.43689207	sperm	q2w
-0.906555821	sperm	q2w
-0.44851741	sperm	q2w
-0.306688258	sperm	q2w
-0.127658016	sperm	q2w
0.13042454	sperm	q2w
0.062997566	sperm	q2w
0.142049881	sperm	q2w
0.121124268	sperm	q2w
-0.120682812	sperm	q2w
-0.36481496	sperm	q2w
-0.216010603	homo	q2w
0.207151787	homo	q2w
0.19087631	homo	q2w
0.258303284	homo	q2w
0.165300561	homo	q2w
-0.408991253	homo	q2w
0.090898383	homo	q2w
0.018821273	homo	q2w
-0.102082267	homo	q2w
-0.197410058	homo	q2w
-0.164859105	homo	q2w
0.056022362	homo	q2w
0.016496205	homo	q2w
0.346655871	homo	q2w
0.195526446	homo	q2w
0.093223451	homo	q2w
0.132749608	homo	q2w

0.755867852	homo	q2w
0.074622906	homo	q2w
0.167625629	homo	q2w
0.067647702	homo	q2w
-0.067206246	homo	q2w
-0.248561556	homo	q2w
0.137399744	homo	q2w
0.36060628	homo	q2w
-0.360164824	homo	q2w
-0.129983084	homo	q2w
-0.181134582	homo	q2w
0.541961589	homo	q2w
0.639614448	homo	q2w
0.655889925	homo	q2w
0.435008458	homo	q2w
0.342005735	homo	q2w
-0.132308152	homo	q2w
0.165300561	homo	q2w
0.332705463	homo	q2w
-0.171834309	homo	q2w
0.439658594	homo	q2w
0.093223451	homo	q2w
-0.060231042	homo	q2w
0.024140604	control-	q2w
-0.018556399	control-	q2w
-0.006931059	control-	q2w
-0.025034944	control-	q2w
0.002596357	control-	q2w
0.0376371	control-	q2w
-0.038231519	control-	q2w
0.024379858	control-	q2w
0.602836879	sperm	q4w
-0.169795578	sperm	q4w
0.14226116	sperm	q4w
0.309136421	sperm	q4w
0.41093033	sperm	q4w
0.337505215	sperm	q4w
0.280767626	sperm	q4w
0.627868169	sperm	q4w
0.087192324	sperm	q4w
-0.393408427	sperm	q4w
0.352523988	sperm	q4w
0.157279933	sperm	q4w
-0.341677096	sperm	q4w
-0.113057989	sperm	q4w
-0.189820609	sperm	q4w
0.028785982	sperm	q4w
0.195661243	sperm	q4w
0.182311222	sperm	q4w
-0.513558615	sperm	q4w
0.043804756	sperm	q4w
0.217355027	sperm	q4w
0.212348769	sperm	q4w
0.048811014	sperm	q4w
-0.745515227	sperm	q4w
0.48769295	sperm	q4w

0.073842303	sperm	q4w
-0.386733417	sperm	q4w
-0.221526909	sperm	q4w
-0.251564456	sperm	q4w
0.280767626	sperm	q4w
0.170629954	homo	q4w
0.289111389	homo	q4w
0.229036295	homo	q4w
0.113892365	homo	q4w
-0.034626617	homo	q4w
-0.021276596	homo	q4w
0.130579892	homo	q4w
0.017104714	homo	q4w
-0.073007927	homo	q4w
-0.009595327	homo	q4w
0.200667501	homo	q4w
0.167292449	homo	q4w
0.135586149	homo	q4w
-0.021276596	homo	q4w
0.053817272	homo	q4w
-0.071339174	homo	q4w
0.192323738	homo	q4w
-0.0880267	homo	q4w
0.022110972	homo	q4w
0.138923655	homo	q4w
0.18731748	homo	q4w
-0.189820609	homo	q4w
0.242386316	homo	q4w
-0.188151856	homo	q4w
-0.12640801	homo	q4w
0.11055486	homo	q4w
0.098873592	homo	q4w
0.118898623	homo	q4w
0.183979975	homo	q4w
0.112223613	homo	q4w
0.265748853	homo	q4w
0.377555277	homo	q4w
0.247392574	homo	q4w
0.21902378	homo	q4w
0.287442637	homo	q4w
0.304130163	homo	q4w
0.350855236	homo	q4w
0.280767626	homo	q4w
0.335836462	homo	q4w
0.284105131	homo	q4w
-0.019607843	control-	q4w
-0.018940342	control-	q4w
0.031122236	control-	q4w
0.007425949	control-	q4w
0	control-	q4w

Script 1. R code for the analyses of the data.

```
##############
#### R code ####
##############

# Useful functions:
# *****************
sidak <- function (p, n) {
  P <- 1-(1-p)^n
  return(P)
}
source("anova.2way.unbalanced.R")
source("t.perm.R")

# Data:
#******
data  <- read.table("data.txt",  h = TRUE, sep = " ")
dataf <- read.table("dataf.txt", h = TRUE, sep = " ")

# Do the negative control differ from 0?
# *****************************************
control <- subset(data, tissue == "c-")
mod_cont <- lm(ABA ~ 1, data = control)
summary(mod_cont)

# I. General significance test of the model: comparisons between females
# *********************************************
test <- anova.2way.unbalanced(dataf$ABA, dataf$tissue, dataf$ind, model="direct",
nperm = 9999,                                         strata=FALSE, silent=FALSE)
test

# II. Multiple comparisons by permutations:
# ********************************************
# We create a list for the dataframe subsets. The subsets are combinations of levels of
our two
# factors.
listdata <- vector("list", 18)
names(listdata) <- c("homo_male", "homo_gyne", "homo_q1d", "homo_q1w",
"homo_q2w", "homo_q4w",  "sperm_male", "sperm_gyne", "sperm_q1d", "sperm_q1w",
"sperm_q2w", "sperm_q4w","control_male", "control_gyne", "control_q1d",
"control_q1w", "control_q2w", "control_q4w")

homo <- subset(data, tissue == "homo")
listdata[[1]] <- subset(homo, ind == "male")
listdata[[2]] <- subset(homo, ind == "gyne")
listdata[[3]] <- subset(homo, ind == "q1d")
listdata[[4]] <- subset(homo, ind == "q1w")
listdata[[5]] <- subset(homo, ind == "q2w")
listdata[[6]] <- subset(homo, ind == "q4w")
```

```
sperm <- subset(data, tissue == "sperm")
listdata[[7]] <- subset(sperm, ind == "male")
listdata[[8]] <- subset(sperm, ind == "gyne")
listdata[[9]] <- subset(sperm, ind == "q1d")
listdata[[10]] <- subset(sperm, ind == "q1w")
listdata[[11]] <- subset(sperm, ind == "q2w")
listdata[[12]] <- subset(sperm, ind == "q4w")
control <- subset(data, tissue == "c-")
listdata[[13]] <- subset(control, ind == "male")
listdata[[14]] <- subset(control, ind == "gyne")
listdata[[15]] <- subset(control, ind == "q1d")
listdata[[16]] <- subset(control, ind == "q1w")
listdata[[17]] <- subset(control, ind == "q2w")
listdata[[18]] <- subset(control, ind == "q4w")
# Matrix of comparisons defining the elements of listdata to be compared:
matcomp <- matrix(c(1, 13,
                2, 14,
                3, 15,
                4, 16,
                5, 17,
                6, 18,
                7, 13,
                8, 14,
                9, 15,
                10, 16,
                11, 17,
                12, 18,
                1, 7,
                2, 8,
                3, 9,
                4, 10,
                5, 11,
                6, 12,
                2, 3,
                3, 4,
                4, 5,
                5, 6,
                8, 9,
                9, 10,
                10, 11,
                11, 12), ncol = 2, byrow = TRUE)
row.names(matcomp) <- c("homo_male-control", "homo_gyne-control", "homo_q1d-
control",
                  "homo_q1w-control", "homo-q2w_control", "homo q4w_control",
                  "sperm male_control", "sperm gyne_control", "sperm q1d_control",
                  "sperm q1w_control", "sperm q2w_control", "sperm q4w_control",
                  "homo_male - sperm_male", "homo_gyne - sperm_gyne",
                  "homo_q1d - sperm_q1d", "homo_q1w - sperm_q1w",
                  "homo_q2w - sperm_q2w", "homo_q4w - sperm_q4w",
                  "homo_gyne - homo_q1d", "homo_q1d - homo_q1w",
```

```
                        "homo_q1w - homo_q2w", "homo_q2w - homoq4w",
                        "sperm_gyne - sperm_q1d", "sperm_q1d - sperm_q1w",
                        "sperm_q1w - sperm_q2w", "sperm_q2w - spermq4w")

# We create a result matrix containing three columns: 'tref' for the observed t.Student

# statistic values, 'p_uncorr' for the uncorrected p-value as obtained with the
permutation
# test, and 'p_corr' for the p-value after performing a Sidak correction for multiple
# comparisons (here, nb of tests = 30). The latter prevents an inflation of the type I error

# rate and is therefore necessary.
matresults <- matrix(ncol = 3, nrow = nrow(matcomp))
colnames(matresults) <- c("t.ref","p_uncorr", "p_corr")
row.names(matresults) <- row.names(matcomp)
for (i in 1:nrow(matcomp)) {
    t <- t.perm(listdata[[matcomp[i, 1]]]$ABA, listdata[[matcomp[i, 2]]]$ABA, nperm =
9999,
                silent = TRUE)
  matresults[i, 1] <- t$t.ref
  matresults[i, 2] <- t$p.perm
  matresults[i, 3] <- sidak(t$p.perm, nrow(matcomp))
}
write.table(matresults, file = "Results - Mult. comparisons.txt", sep = "\t")
```