

Supplemental materials

Survival trends of combined colonies

Age of first foraging

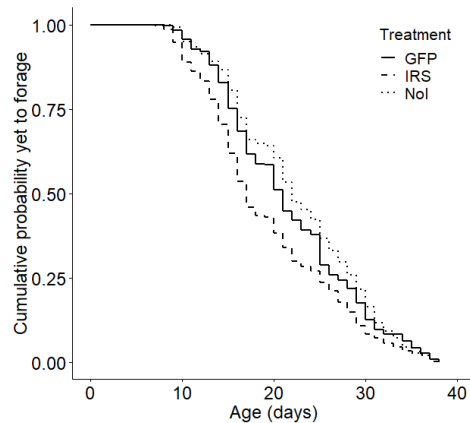


Figure S1. Effect of IRS *irs* knockdown on age of foraging onset for all bees. There was a significant overall effect of treatment on age of foraging onset (Kaplan-Meier: $\chi^2 = 33.089$, $p < 0.0001$; $n = \text{irs knockdown (IRS):287, GFP injected control (GFP):302, non-injected reference (NoI):305}$). IRS *irs* knockdown induced an early onset of foraging behavior relative to injected GFP controls (Cox-Mantel: $U = -34.759$, $p < 0.002$). There was no effect of handling or injection stress on age of first foraging (Cox-Mantel: $U = 14.607$, $p = 0.206$).

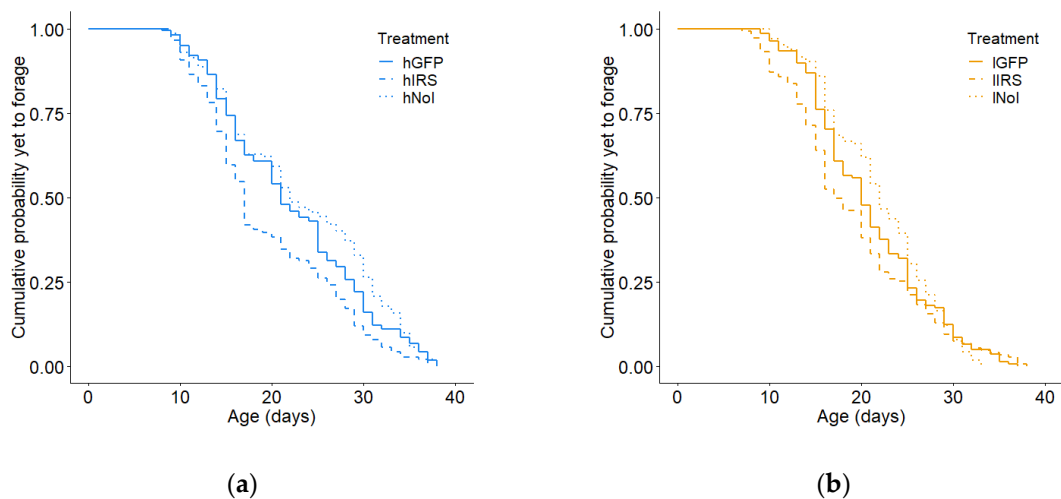


Figure S2. Effect of *irs* knockdown on age of first foraging by strain. a) We observed an overall effect of treatment in the high strain (Kaplan-Meier: $\chi^2 = 16.166$, $p < 0.002$; $n = \text{high strain } \textit{irs}$ knockdown (hIRS):140, high strain GFP injected control (hGFP):163, high strain non-injected reference (hNoI):140), and *irs* knockdown induced early foraging onset in high strain workers (Cox-Mantel: $U = 22.845$, $p < 0.004$). b) While there was an overall effect of treatment in the low strain Kaplan-Meier: $\chi^2 = 18.044$, $p = 0.0001$; $n = \text{low strain } \textit{irs}$ knockdown (IIRS):147, low strain GFP injected control (IGFP):139, low strain non-injected reference (INoI):165), *irs* knockdown had no significant effect on the age of foraging onset in this strain (Cox-Mantel: $U = -4.468$, $p = 0.234$).

Total lifespan

Total lifespan was reduced by *irs* knockdown (Kaplan-Meier: $\chi^2 = 23.178$, $p < 0.0001$; $n = \text{IRS}:618$, $\text{GFP}:665$, $\text{NoI}:688$; Cox-Mantel: $U = 88.349$, $p < 0.0001$; Figure S3). Handling stress and injection of dsRNA in general had no effect on total lifespan (Cox-Mantel: $U = 4.567$, $p = 0.767$; Figure S3). Further, *irs* knockdown reduced lifespan in both the high strain (Kaplan-Meier: $\chi^2 = 14.21$, $p = 0.005$; $n = \text{hIRS}:320$, $\text{hGFP}:351$, $\text{hNoI}:378$; Cox-Mantel: $U = -48.724$, $p < 0.0001$; Figure S4a) and the low strain (Kaplan-Meier: $\chi^2 = 12.927$, $p = 0.0016$; $n = \text{lIRS}:298$, $\text{lGFP}:314$, $\text{lNoI}:310$; Cox-Mantel: $U = -33.646$, $p < 0.0008$; Figure S4b). There was no effect of handling and injection of lifespan in either strain (High strain: Cox-Mantel: $U = 10.485$, $p = 0.346$; Low strain: $U = -18.915$, $p = 0.0711$; Figure S4a,b).

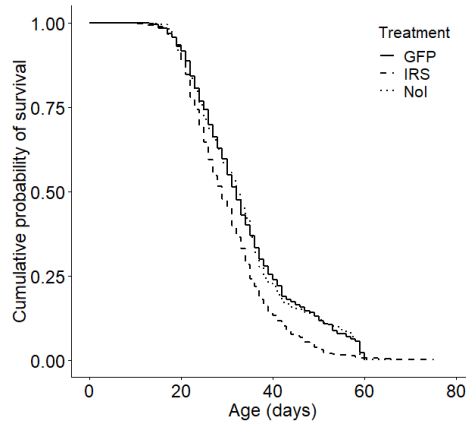


Figure S3. Effect of *irs* knockdown on total lifespan for all bees. There was an overall effect of treatment on total lifespan for all bees (Kaplan-Meier: $\chi^2 = 23.178$, $p < 0.0001$; $n = \text{irs knockdown (IRS):618}$, $\text{GFP injected controls (GFP):665}$, $\text{non-injected reference (NoI):688}$). *irs* knockdowns had significantly shorter lives than *GFP* (Cox-Mantel: $U = 88.349$, $p < 0.0001$). There was no significant effect of handling or injection on total lifespan (Cox-Mantel: $U = 4.567$, $p = 0.767$).

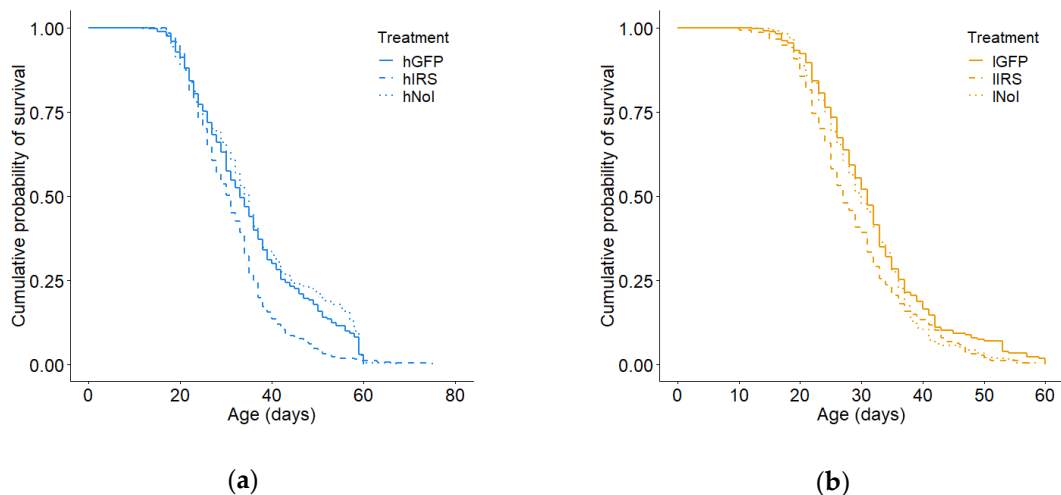


Figure S4. Effect of *irs* knockdown on total lifespan by strain. A) In the high strain, *irs* knockdown resulted in decreased lifespan relative to the *GFP* control (Kaplan-Meier: $\chi^2 = 14.21$, $p = 0.005$; $n = \text{high strain } \text{irs knockdown (hIRS):320}$, $\text{high strain GFP injected control (hGFP):351}$, $\text{high strain non-injected reference (hNoI):378}$; Cox-Mantel: $U = -48.724$, $p < 0.0001$). There was no effect of handling on total lifespan in the high strain (Cox-Mantel: $U = 10.485$, $p = 0.346$). B) In the low strain, *irs* knockdown also induced decreased lifespan relative to *GFP* controls (Kaplan-Meier: $\chi^2 = 12.927$, $p = 0.0016$; $n = \text{low strain } \text{irs knockdown (lIRS):298}$, $\text{low strain GFP injected control (lGFP):314}$, $\text{low strain non-injected reference (lNoI):310}$; Cox-Mantel: $U = -33.646$, $p < 0.0008$). We again observed no effect of handling on lifespan in this strain (Cox-Mantel: $U = -18.915$, $p = 0.0711$).

In contrast to both age of first foraging and total lifespan, foraging lifespan, the length of time between the first foraging flight and death, was not affected by irs knockdown (Kaplan-Meier: $\chi^2 = 2.480$, $p = 0.289$; $n = \text{IRS:}285, \text{GFP:}301, \text{NoI:}305$). irs knockdown did not affect foraging lifespan in either the high strain (Kaplan-Meier: $\chi^2 = \chi^2 = 3.596$, $p = 0.166$; $n = \text{hIRS:}140, \text{hGFP:}163, \text{hNoI:}140$) or the low strain (Kaplan-Meier: $\chi^2 = 0.1333$, $p = 0.9355$; $n = \text{lIRS:}144, \text{lGFP:}138, \text{lNoI:}165$).

Table 1. Descriptive statistics for age of first foraging and lifespan components data.

| Age of First Foraging | | | | | | |
|------------------------------|-----------|-----|----------|--------------------|------------|-------------------------|
| Strain | Treatment | N | Mean (d) | Standard Error (d) | Median (d) | Interquartile Range (d) |
| High pollen hoarding | GFP | 163 | 22.15 | 0.62 | 21 | 14 |
| | IRS | 141 | 19.40 | 0.63 | 17 | 12 |
| | NoI | 140 | 23.24 | 0.72 | 22 | 16 |
| Low pollen hoarding | GFP | 138 | 20.92 | 0.56 | 20 | 9 |
| | IRS | 147 | 19.17 | 0.60 | 17 | 10.5 |
| | NoI | 165 | 21.96 | 0.47 | 22 | 10 |
| Lifespan | | | | | | |
| Strain | Treatment | N | Mean (d) | Standard Error (d) | Median (d) | Interquartile Range (d) |
| High pollen hoarding | GFP | 351 | 32.25 | 0.69 | 30 | 17 |
| | IRS | 320 | 29.42 | 0.55 | 28 | 13 |
| | NoI | 378 | 30.91 | 0.72 | 27.5 | 20 |
| Low pollen hoarding | GFP | 314 | 28.65 | 0.56 | 27 | 13 |
| | IRS | 298 | 25.81 | 0.52 | 24 | 11 |
| | NoI | 310 | 28.46 | 0.48 | 27 | 12.75 |
| Foraging Lifespan | | | | | | |
| Strain | Treatment | N | Mean (d) | Standard Error (d) | Median (d) | Interquartile Range (d) |
| High pollen hoarding | GFP | 163 | 10.16 | 0.67 | 8 | 10 |
| | IRS | 141 | 11.40 | 0.72 | 10 | 11 |
| | NoI | 140 | 10.24 | 0.73 | 7 | 11 |
| Low pollen hoarding | GFP | 138 | 8.12 | 0.58 | 7 | 8.75 |
| | IRS | 144 | 8.14 | 0.52 | 7 | 7 |
| | NoI | 165 | 7.56 | 0.39 | 7 | 8 |