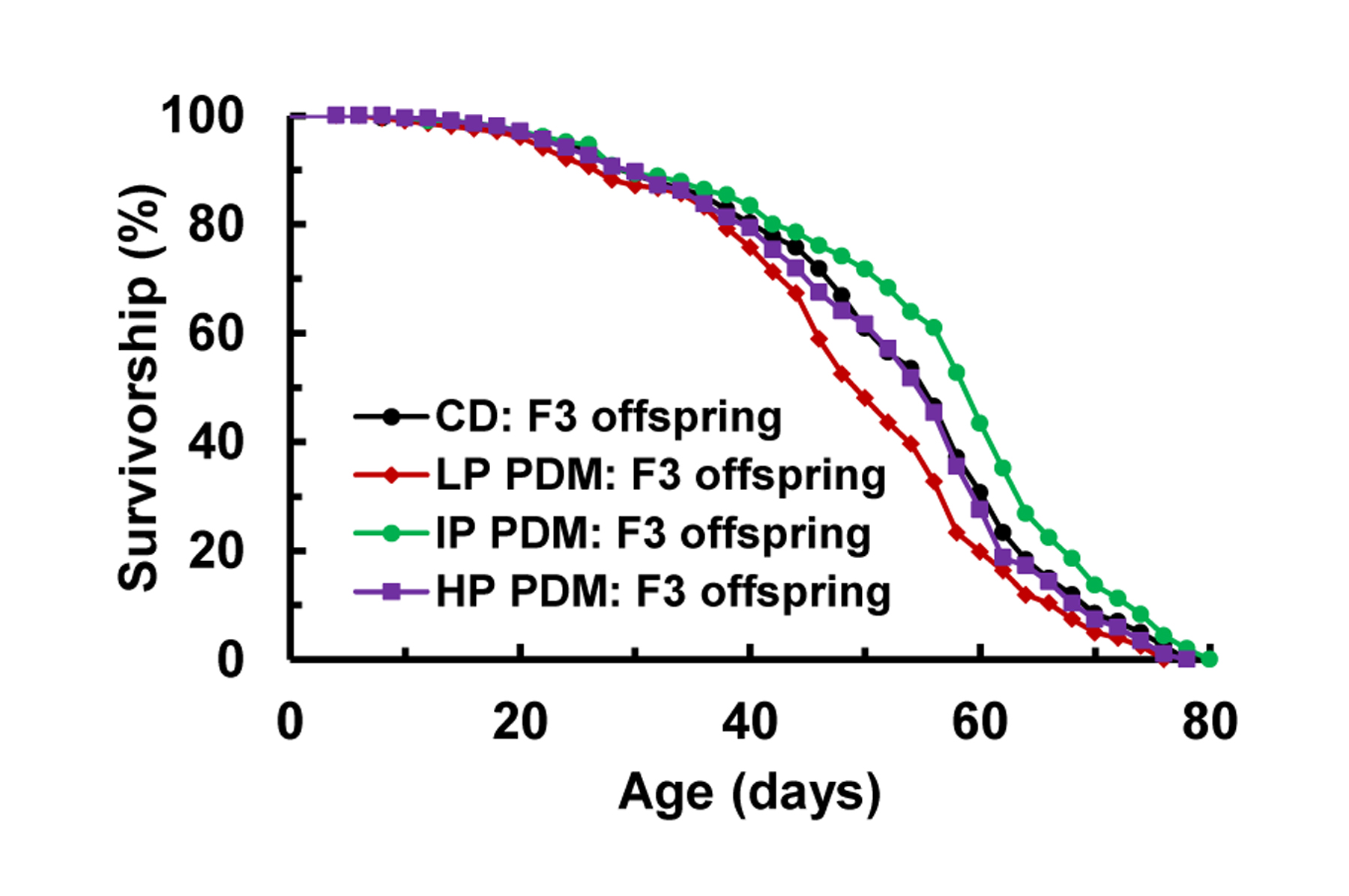
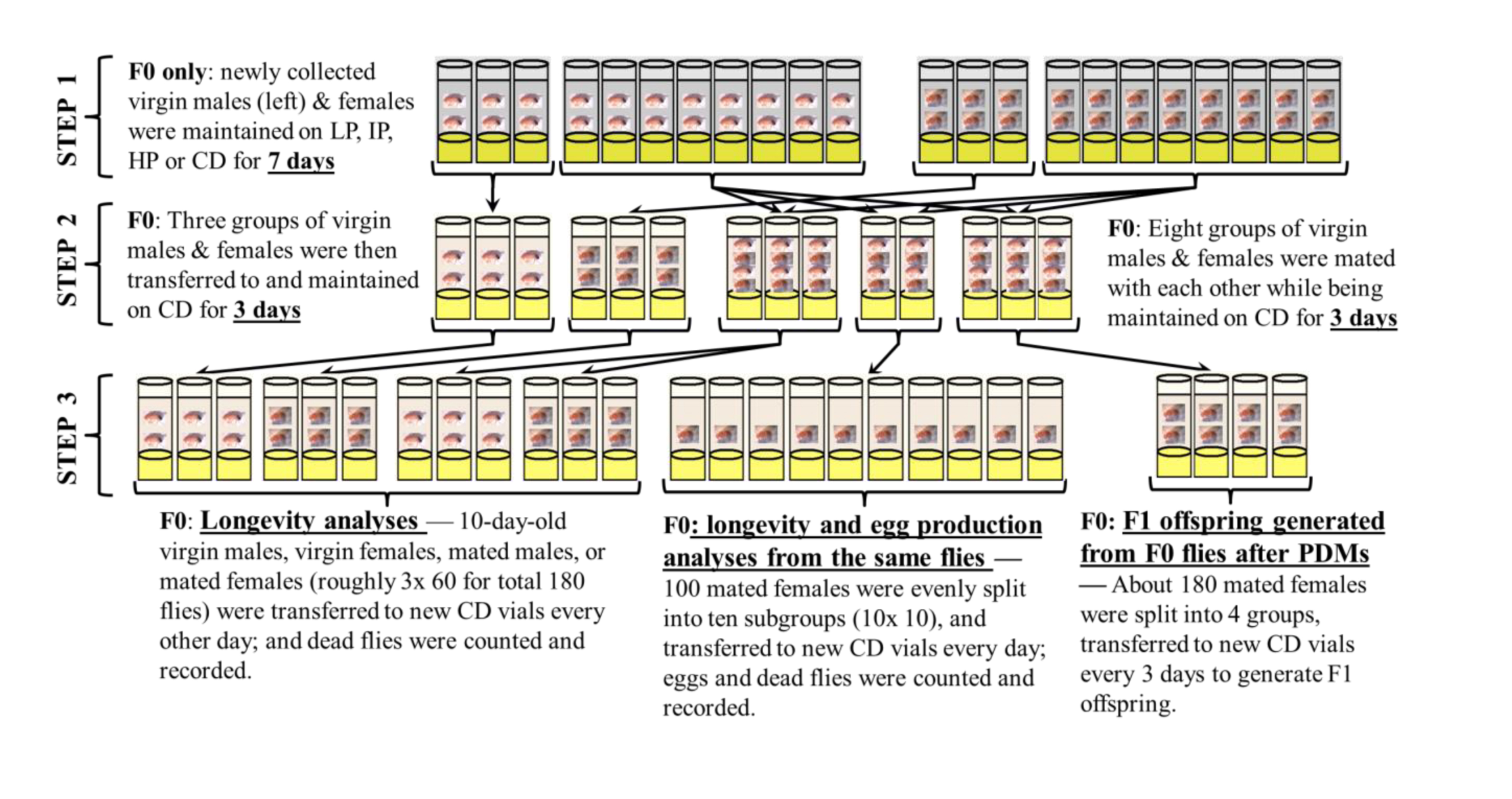
**SUPPLEMENTAL FIGURES**



**Supplemental Figure S2**. Survival curves for the F3 generation (mated females only). The LP PDM of the F0 flies shortened longevity of their F3 offspring significantly (P = 0.003; Mantel-Cox test), or by 11% at the median lifespan (50 vs. 56 for LP vs. CD); while the IP PDM of the F0 flies improved longevity of their F3 offspring (P = 0.005), or 7% at the median lifespan (60 vs. 56 for IP vs. CD). The HP PDM of F0 flies induced no effect on F3 offspring any further (P = 0.46; 56 vs. 56 for median lifespan).

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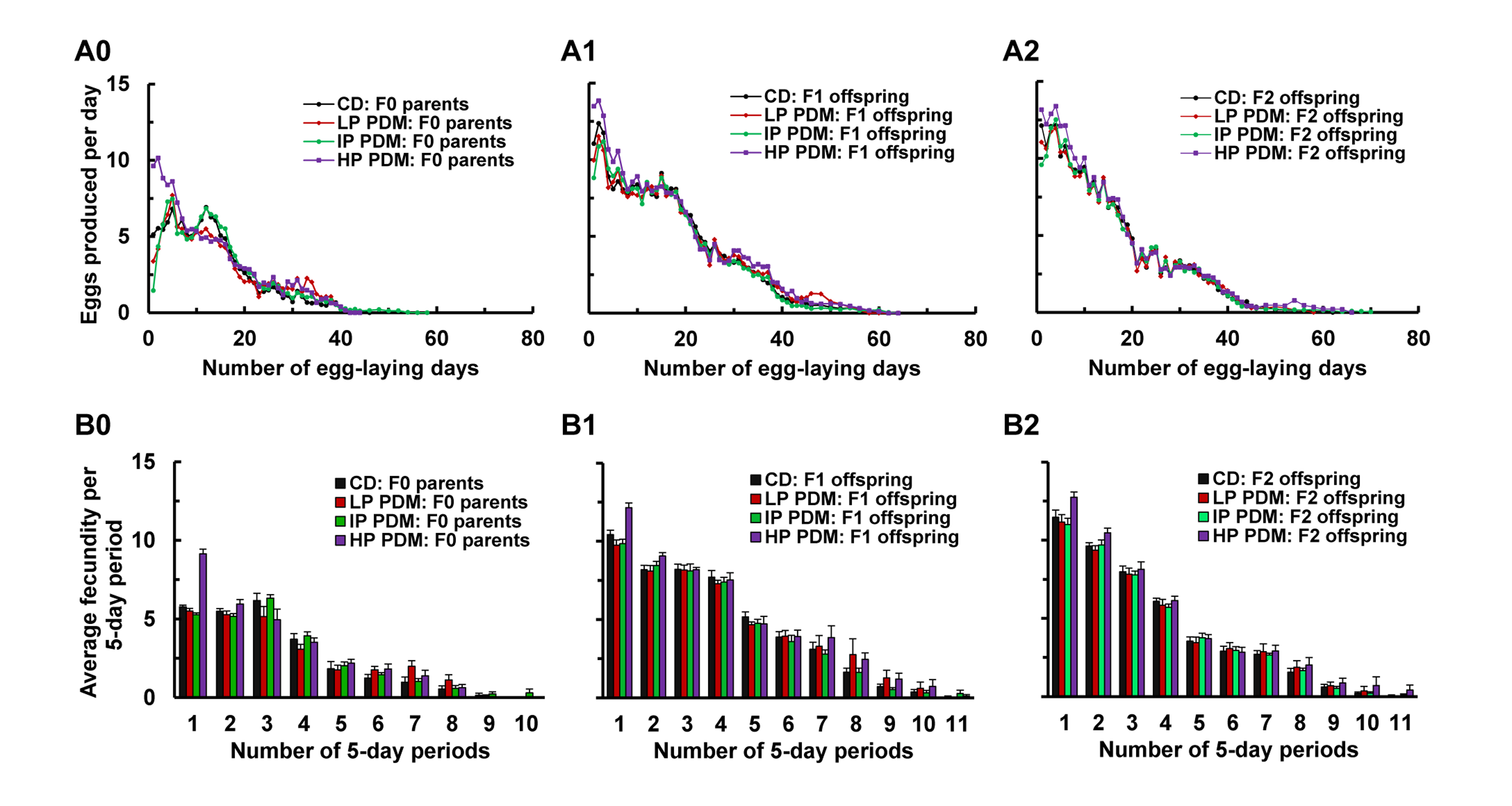
**SUPPLEMENTAL FIGURES**



**Supplemental Figure S1.** Experimental design and procedures. Shown here is the F0 generation, subjected to the 7-day PDMs before longevity and fecundity analyses. **STEP 1**) Virgin males and females were collected and subjected to 7-day PDMs. **STEP 2**) Three groups of 60 virgin males and females were transferred to CD for 3 days, while the others (8 groups) were mated with each other for 3 days on CD. **STEP 3**) *Longevity analyses:* three groups (~180 flies) of virgin males, virgin females, mated males, and mated females were used; *egg production and longevity analyses from same flies:* 100 mated females were evenly split into 10 subgroups (10x10) and used for analyses; *F1 offspring generated from the F0 flies after PDMs:* 180 mated females were split into 4 groups and used for generating the F1 offspring while being maintained on CD all the time. Similar analyses were done with their F1, F2, and F3 offspring, by repeating **STEPs 2−3** (i.e., without PDMs from **STEP 1**) as for the F0 parents while using newly-born virgin males and females, except that roughly 4x 50 flies were used for longevity analyses of the F2−F3 offspring.

**Supplemental Figure S3**. Eggs produced per day and 5-day period. (**A**) Average number of “*Eggs Produced per Day*” (1st row) and (**B**) “*Fecundity per 5-day Period*” (2nd row); and (**0**) F0 parents (1st column), (**1**) F1 offspring (2nd column), and (**2**) F2 offspring (3rd column) by the 100 mated females. “Eggs Produced per Day” was calculated as “total eggs produced for a given day divided by the number (1–100) of the surviving flies within a diet group”. “Fecundity per 5-day Period” was defined as the average number of eggs laid over a 5-day period by one mated female.

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**SUPPLEMENTAL TABLES**

**Supplemental Table S1.** Diets used for the PDMs of the F0 parents.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Ingredients** | **Control diet (CD) A** | **LP diet B** | **IP diet C** | **HP diet D** |
| **Recipes** | Yellow cornmeal (gm) | 76.6 | 98.0 | 92.0 | 162.5 |
| Yeast (gm) | 32.1 |  | 17.2 | 33.5 |
| Agar (gm) | 9.3 | 10.0 | 5.2 | 7.5 |
| Glucose (gm) | 63.2 | 150.0 | 147.4 |  |
| Sucrose (gm) | 31.6 |  |  | 40.0 |
| CaCl2 (gm) | 0.7 |  |  |  |
| Soy flour (gm) |  |  |  | 30.0 |
| Water (gm) | 1000 | 1000 | 1000 | 1000 |
| **Total (gm, with water)** | | 1213.5 | 1258.0 | 1261.8 | 1273.5 |
| **Protein (%; with water) E** | | 8.6 | 3.3 | 5.5 | 13.5 |
| **Carbohydrate (%; with water) E** | | 76.6 | 90.5 | 87.4 | 69.6 |
| **Total calories E** | | 758.0 | 969.4 | 973.6 | 982.6 |
| **Calories per gram F** | | 0.62 | **0.77** | **0.77** | **0.77** |

Provided are the recipes, along with the protein, carbohydrate, and calorie information for the control diet (CD) and 3 other diets used for the PDMs of the F0 parents. (**A**) CD is a food medium routinely used in the lab. (**B**) The “LP” (Low Protein) diet was adapted from Xia et al [28]. (**C**) The “IP” (Intermediate Protein) diet was adapted from Guo et al [27]. (**D**) The “HP” (High Protein) diet was adapted from a widely used “standard diet” as described by Guo et al [27] and at Bloomington Stock Center (<http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/bloomfood.htm>). (**E**) The protein, carbohydrate, and calorie information for all the ingredients has been obtained from their labels or from <http://nutritiondata.self.com/>. (**F**) All three diet recipes were slightly modified to be isocaloric (0.77 calories/gm food).

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**Supplemental Table S2.** Sample size (N) for four types of flies after each PDM across the F0−F3 generations in the longevity experiments.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **PDM diets** | **F0 parents (**+ PDM**)** | **F1 offspring** | **F2 offspring** | **F3 offspring** |
| **Virgin males** | CD (control) | 181 | 184 | 198 | − |
| LP (F0 only) | 183 | 182 | 202 | − |
| IP (F0 only) | 180 | 181 | 200 | − |
| HP (F0 only) | 182 | 180 | 201 | − |
| **Virgin females** | CD (control) | 180 | 183 | 201 | − |
| LP (F0 only) | 182 | 182 | 202 | − |
| IP (F0 only) | 184 | 178 | 203 | − |
| HP (F0 only) | 180 | 185 | 201 | − |
| **Mated males** | CD (control) | 181 | 180 | 199 | − |
| LP (F0 only) | 182 | 181 | 200 | − |
| IP (F0 only) | 179 | 181 | 201 | − |
| HP (F0 only) | 180 | 185 | 202 | − |
| **Mated females** | CD (control) | 185 | 183 | 199 | 202 |
| LP (F0 only) | 183 | 181 | 203 | 202 |
| IP (F0 only) | 182 | 183 | 200 | 205 |
| HP (F0 only) | 181 | 182 | 201 | 203 |
| **Mated females (**same flies also assayed for reproduction**)** | CD (control) | 100 | 100 | 100 | − |
| LP (F0 only) | 100 | 100 | 100 | − |
| IP (F0 only) | 100 | 100 | 100 | − |
| HP (F0 only) | 100 | 100 | 100 | − |
| **Subtotal (**per generation**)** | | **3305** | **3311** | **3613** | **812** |
| **Total (**whole study**)** | |  | **11,041** |  |  |

**Supplemental Table S3.** Number of eggs recorded for each subgroup (10 flies) of the mated females from the F0 parents (with PDM) and their F1-F2 offspring.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PDM diets** |  | | **F0 parents (**+ PDM**)** | | **F1 offspring** | | **F2 offspring** |
| **Mated females (**10x subgroups of 10 flies; same flies also assayed for longevity**)** | **CD** (control) | Subgroup 1 | | 1101 | | 2037 | | 2022 |
| Subgroup 2 | | 1139 | | 2057 | | 1961 |
| Subgroup 3 | | 1070 | | 2072 | | 2095 |
| Subgroup 4 | | 1192 | | 2111 | | 1982 |
| Subgroup 5 | | 1213 | | 2040 | | 1987 |
| Subgroup 6 | | 1088 | | 2253 | | 2060 |
| Subgroup 7 | | 1122 | | 2096 | | 2027 |
| Subgroup 8 | | 1034 | | 2132 | | 2096 |
| Subgroup 9 | | 1017 | | 2123 | | 2061 |
| Subgroup 10 | | 957 | | 2160 | | 2118 |
| Mean ± SEM | | 1093.3 ± 24.8 | | 2108.1 ± 20.6 | | 2040.9 ± 17.0 |
| Total per generation | | 10933 | | 21081 | | 20409 |
| **LP** (F0 only) | Subgroup 1 | | 916 | | 2008 | | 1854 |
| Subgroup 2 | | 902 | | 1950 | | 1796 |
| Subgroup 3 | | 769 | | 1794 | | 1926 |
| Subgroup 4 | | 899 | | 1946 | | 1947 |
| Subgroup 5 | | 1015 | | 1853 | | 1811 |
| Subgroup 6 | | 825 | | 1891 | | 1923 |
| Subgroup 7 | | 873 | | 1719 | | 1867 |
| Subgroup 8 | | 902 | | 1996 | | 1704 |
| Subgroup 9 | | 834 | | 1859 | | 1908 |
| Subgroup 10 | | 1014 | | 1716 | | 1731 |
| Mean ± SEM | | 894.9 ± 24.5 | | 1873.2 ± 33.4 | | 1846.7 ± 26.7 |
| Total per generation | | 8949 | | 18732 | | 18467 |
| **IP** (F0 only) | Subgroup 1 | | 1095 | | 2150 | | 2128 |
| Subgroup 2 | | 1287 | | 2315 | | 2165 |
| Subgroup 3 | | 1100 | | 2252 | | 2115 |
| Subgroup 4 | | 1127 | | 2176 | | 2089 |
| Subgroup 5 | | 1336 | | 2205 | | 2152 |
| Subgroup 6 | | 1187 | | 2348 | | 2196 |
| Subgroup 7 | | 1074 | | 2168 | | 2199 |
| Subgroup 8 | | 1242 | | 2222 | | 2161 |
| Subgroup 9 | | 1161 | | 2062 | | 1942 |
| Subgroup 10 | | 1237 | | 2211 | | 2213 |
| Mean ± SEM | | 1184.6 ± 28.0 | | 2210.9 ± 25.9 | | 2136.0 ± 24.8 |
| Total per generation | | 11846 | | 22109 | | 21360 |
| **HP** (F0 only) | Subgroup 1 | | 1061 | | 2288 | | 2154 |
| Subgroup 2 | | 1214 | | 2148 | | 2163 |
| Subgroup 3 | | 1180 | | 2168 | | 2130 |
| Subgroup 4 | | 1083 | | 2163 | | 2256 |
| Subgroup 5 | | 1321 | | 2199 | | 2177 |
| Subgroup 6 | | 1140 | | 2292 | | 1952 |
| Subgroup 7 | | 1121 | | 2132 | | 2183 |
| Subgroup 8 | | 1217 | | 2172 | | 2052 |
| Subgroup 9 | | 1298 | | 2076 | | 2057 |
| Subgroup 10 | | 1100 | | 2257 | | 1992 |
| Mean ± SEM | | 1173.5 ± 28.1 | | 2189.5 ± 22.2 | | 2111.6 ± 30.1 |
| Total per generation | | 11735 | | 21895 | | 21116 |
| **Subtotal (**per generation across four diets**)** | | | **43463** | | **83817** | | **81352** | |
| **Total (**whole study**)** | | | **208,632** | | | | | |

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