Methods:

Insect rearing:

*Ostrinia nubilalis* eggs were taken from colonies at Tufts University. The two genetically distinct strains used for this work were initially collected as a mixture of larvae, pupae, and adults from New York State prior to 2015 and kept as separate colonies (Wadsworth et al., 2015). Strain identity was determined genotypically using the *pgFAR* (Lassance et al., 2010), an autosomal locus coding for a critical enzyme for determining the female sex pheromone blends that play an important role along with seasonal timing in reproductively isolating these two strains from each other in the field (Dopman et al. 2010). For the duration of the experiment, colonies of each genotype were reared at the University of Florida at 26°C under a 16L:8D photoperiod to promote continuous development.

Comparing the timing of diapause initiation between strains:

Larval diapause in European corn borer begins after the termination of larval feeding, during the so called wandering stage, and diapause ends when larvae undergo larval-pupal metamorphosis (refs). To determine whether the timing of larval feeding, pupation, and diapause initiation differed between the two strains, we observed feeding in last-instar larvae of both continuously developing and diapause-programmed larvae of each strain. To assay when feeding ended, synchronized cohorts of individuals were taken from eggs laid by multiple females from each strain on a single day. Upon hatching, each cohort was divided and reared in either a diapause-inducing treatment (12L:12D photoperiod, 23°C, and 65% rH) or a non-diapause treatment that promoted continuous development of larvae into pupae (16L:8D photoperiod, 23°C, and 65% rH). Larvae from each biological cohort were reared together in mass and provided artificial European corn borer diet ad libitum (Frontier Agricultural Sciences, Newark, DE, Product F9478B). When larvae from each biological cohort within each treatment reached the end of the fourth instar, they were separated and reared individually in 32-well bioassay trays for the last larval instar (Frontier Agricultural Sciences, Newark DE, Product RT32W). Each well of the bioassay tray was provisioned with diet and returned to either diapause-inducing or non-diapause treatment conditions until larvae were sampled. To assess whether an individual caterpillar had completed larval feeding and was transitioning into the wandering stage, each individual larva was removed from their rearing tray and placed into a similar tray that contained no artificial diet. Preliminary experiments revealed that actively feeding larvae always produced frass within 30 minutes of being isolated from food, thus each individual was scored as feeding or not feeding/wandering after 30 minutes.

Comparing lipid and lean mass between strains:

We estimated both fat mass and lean mass for each strain and each life-history trajectory, diapause or non-diapause, on the first day of the last larval instar and on the first day of wandering. We chose these time points to determine whether strain or diapause trajectory affected lean or fat mass 1) at the onset of the last instar, and 2) on the first day of wandering just after larvae stop feeding because fat and lean mass peak in larval insects at this time.

At the time of sampling, larvae were weighed then frozen until further analysis. Larvae from each of the 4 treatments (strain by photoperiod) and developmental stage (day 1 of the final instar and day of wandering) were randomly assigned into extraction blocks of 16 larvae each. Larvae were freeze-dried, weighed, and then were homogenized in pre-weighed 1.5ml microcentrifuge tubes (USA Scientific, Ocala, FL., 34478) using a 3:1 solvent mixture of hexanes and methanol that was allowed to settle. The hexanes layer containing lipids was siphoned away from the methanol layer and collected in pre-weighed 15-mL glass vials (Milipore-Sigma, St. Louis, MO., 27347) and both layers were saved. Lean mass was estimated by drying away the methanol from the solubilized insect tissue using a SpeedVac Concentrator (Thermo Scientific, Waltham, MA., 02451) and weighing the dry tissue powder. To estimate lipid mass, the hexanes were dried away from the lipids using a SpeecVac Concentrator and the dry lipids were weighed.

Statistical Analyses

All statistical analyses were performed using R studio software (version 1.1.383). Wandering day was calculated as the total number of days between molting into the final larval instar and the day frass production ended for each sampled larva. Wandering day was measured in 48 individuals, 12 in each strain x photoperiod combination, and analyzed using a generalized linear mixed effects model. The statistical model to explain differences in wandering day included: diapause genotype and photoperiod as fixed effects, diapause genotype and photoperiod as interacting effects, and cohort as a random factor. Lipid stores were measured in 266 individuals and analyzed using a generalized linear mixed effects model. The statistical model to explain lipid mass prior to the onset of diapause included: diapause genotype and photoperiod as fixed effects, diapause genotype and photoperiod as interacting fixed effects, and lean mass was a covariate. The model to explain lipid mass depletion during diapause included: diapause genotype and sample day as fixed effects, diapause genotype and sample day as interacting fixed effects, and lean mass was a covariate. Lean mass was measured in 338 individuals and analyzed using a generalized linear mixed effects model. The statistical model to explain lean mass prior to the onset of diapause included: diapause genotype and photoperiod as fixed effects and diapause genotype and photoperiod as interacting fixed effects. The model to explain lean mass depletion during diapause included: diapause genotype and sample day as fixed effects and diapause genotype and sample day as interacting fixed effects. Cohort was also included in each generalized linear model as nested within extraction block, and extraction block was used as a random factor.