Methods for construction of the Sleep Inbred Panel (SIP)

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Construction of Sleep Advanced Intercross Population

The process for construction the SIP is outlined in Fig. 1. First, we constructed an outbred population of flies using ten lines from the *Drosophila* Genetic Reference Panel (DGRP) (1, 2) with the most extreme night sleep phenotypes for both sexes combined. Five lines had the shortest average night sleep for both males and females in the population: DGRP_38, DGRP_310, DGRP_365, DGRP_808, and DGRP_832 (3). The other five lines had the longest average night sleep in the population: DGRP_235, DGRP_313, DGRP_335, DGRP_338, and DGRP_379 (3). All ten lines were crossed in a full diallel design, resulting in 100 crosses. Two virgin females and two males from the F1 of each cross were then randomly mixed together and placed into 20 bottles, with 10 males and 10 females in each bottle. At each generation thereafter, 20 virgin females and 20 males from each bottle were randomly mixed with the other flies, and a new generation of 20 bottles was reared. Each generation of random mating had a census population size of 800. The genomes of these flies were allowed to recombine in this manner for 21 generations, resulting in an advanced intercross population of flies we named the Sleep Advanced Intercross Population (SAIP).

Construction of long-sleeping and short-sleeping populations via artificial selection

At Generation 0 of the selection procedure, we divided the SAIP into six populations by seeding four bottles with 25 randomly chosen flies of each sex. Two populations were selected for long night sleep (L1 and L2), and two populations were selected for short night sleep (S1 and S2). We implemented the following artificial selection procedure each generation. First, 100 virgins of each sex were collected from each population bottle and placed into the sleep monitors. Sleep and activity were monitored

continuously over a five-day period. Second, we calculated all sleep parameters for every fly in each selection population. For the four selection populations (L1, L2, S1, and S2), the 25 males and females with the most extreme (high or low) night sleep within each population were chosen as parents for the next generation of that population. We repeated this procedure for 13 generations. After generation 13, the six populations were maintained by seeding culture bottles with 25 randomly chosen flies of each sex.

Construction of the Sleep Inbred Panel (SIP)

At generation 30, the long- and short-sleeping selected populations were used to create inbred lines. We created 10 lines each from the L1, S1, and S2 populations, and 9 lines from the L2 population (39 lines total). Each line was created using a single male and a single female from one of the populations to start the line; full-sib mating continued in this manner for 20 generations. Inbred stocks were maintained past generation 20 by random mating.

1. Mackay TF, Richards S, Stone EA, Barbadilla A, Ayroles JF, Zhu D, et al. The Drosophila melanogaster Genetic Reference Panel. Nature. 2012;482(7384):173-8.
2. Huang W, Massouras A, Inoue Y, Peiffer J, Ramia M, Tarone AM, et al. Natural variation in genome architecture among 205 Drosophila melanogaster Genetic Reference Panel lines. Genome research. 2014;24(7):1193-208.
3. Harbison ST, McCoy LJ, Mackay TF. Genome-wide association study of sleep in Drosophila melanogaster. BMC genomics. 2013;14:281.

Fig. 1. Construction of the Sleep Inbred Panel.