Verifying polymorphisms associated with long and short sleep using polycistronic CRISPR coupled with extreme QTL mapping

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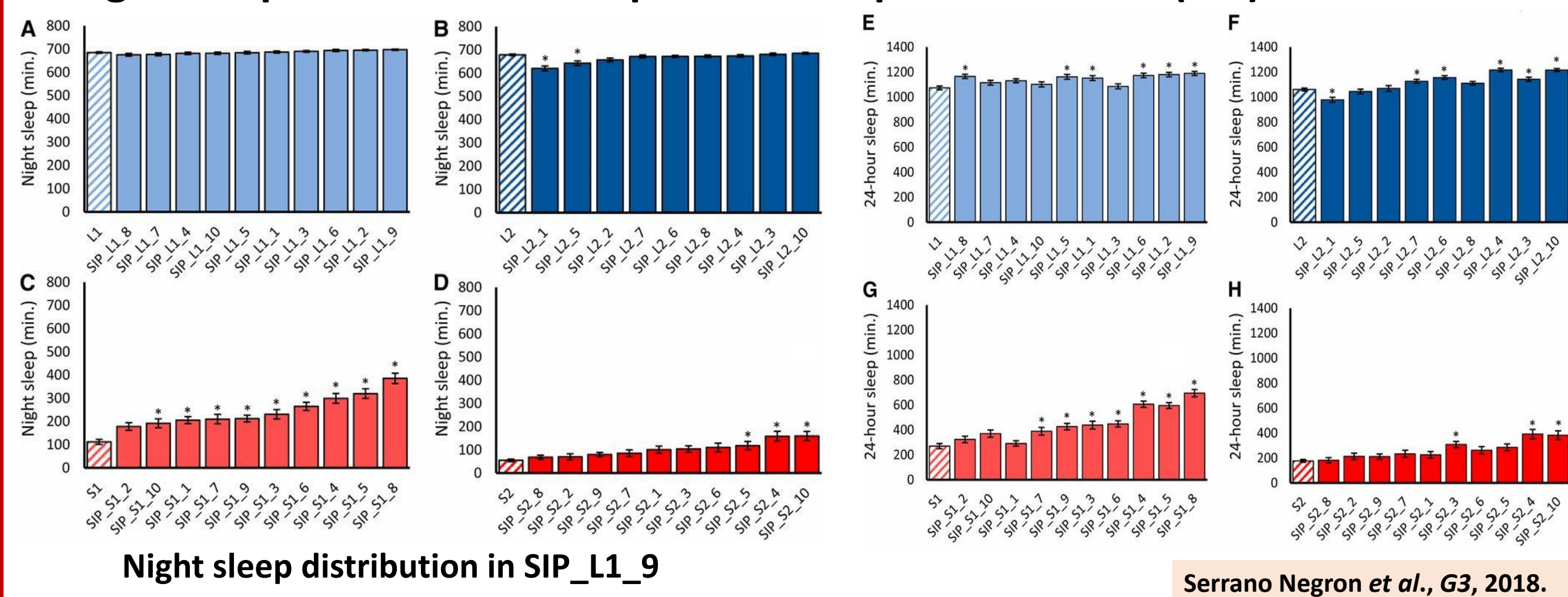[1] Laboratory of Systems Genetics, National Heart Lung and Blood Institute, Bethesda, MD, 20892
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Abstract

Artificial selection and genome-wide association mapping have isolated unprecedented numbers of candidate polymorphisms putatively involved in sleep. One of the greatest challenges in functional genomics is to understand how these polymorphic variants affect sleep. CRISPR technology offers the possibility of replacing alleles to directly observe their effects; however, the task can be daunting for traits having large numbers of predicted polymorphic targets. Previously, we identified 126 polymorphisms for long and short night sleep using artificial selection. Here we apply a new approach to verify these polymorphisms. Using a polycistronic CRISPR gRNA design, we expressed multiple gRNAs per polycistronic construct to create indels near target polymorphisms in a long-sleeping line of the Sleep Inbred Panel, SIP_L1_9. We cloned four gRNAs into a pCFD5 plasmid and injected the plasmid into SIP_L1_9. We allowed the resulting transformants to mate randomly for two generations in order to recombine the transformed chromosomes. We then measured sleep in the transformed populations. Night sleep ranged from 136.7 min. ± 25.2 to 706.3 min. ± 7.4 for progeny from one polycistronic construct and from 266.9 min. ± 103.0 to 696.9 min. ± 9.7 for a second polycistronic construct, exceeding the range of night sleep in the unperturbed SIP_L1_9 background. This suggests that efficient transformation occurred in both populations. We collected the 10% shortest-sleeping and 10% longest-sleeping flies for each construct and extracted their DNA. We are currently sequencing the genomic DNA from each of the high/low 10% and will associate the sleep phenotypes with the number and combination of genomic breaks. In principle, more breaks should be present in the short sleeping flies than in the long sleepers as long sleepers represent the unperturbed background of the injected strain. In this way, we can quickly verify sleep-relevant polymorphisms with the greatest effects, screening out any false positives.

Background

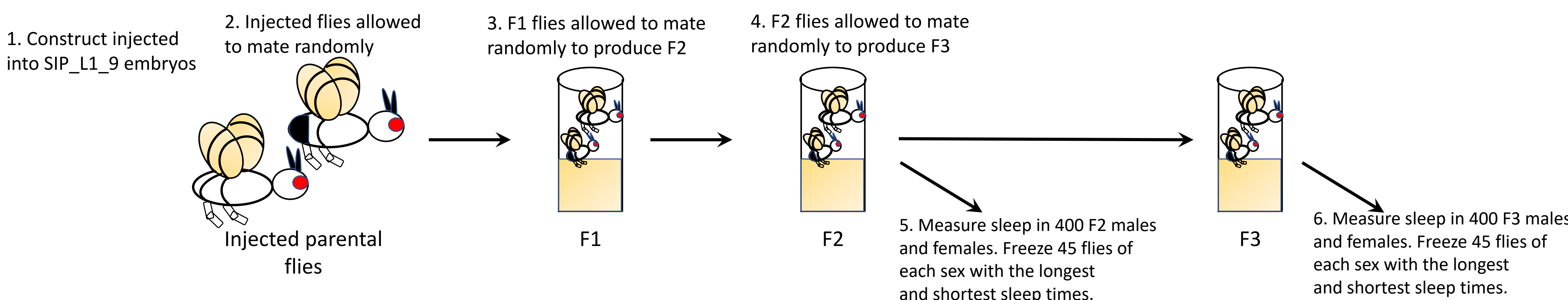### Night sleep and 24-hour sleep in the Sleep Inbred Panel (SIP) Lines

Serrano Negron *et al.*, *G3*, 2018.

Night sleep distribution in SIP_L1_9

Schematic depiction of the polycistronic CRISPR/Cas9 method for simultaneously targeting multiple sites using multiple gRNAs

Port and Bullock, Nat Meth, 2016.

Chromosome *2R* gRNA targets

We designed gRNAs for variants previously associated with long and short sleep (Harbison *et al.*, *PLoS Genetics*, 2017). We used CRISPOR (Haeussler *et al.*, *Genome Biol.*, 2016) and flycrispr (Gratz *et al.*, *Genetics*, 2014) to design the gRNAs. While off-target sites and efficiency calculations were considered, the most important design criterion was proximity to the target variant.

Target variants	Location (bp)	Guide sequence	Polycistronic construct
Intergenic_7	10201855	TGCCACTTTCATCGCAAATG	
CG4744	13393648	ATAGCAATCTATCTCTGAGT	
cbc	13484510	GAAATTTGCAGATTATGAAG	1
CG33156	13550013 13550021	CAATAACAATTGCATTCGGG	
mip120	13506555	CTATGTGCACTTTCATTTGG	
CG33156	13550112	GCGTCCTGATCAGCCGCAGG	2
CG17716	13742025	AAACTTCCGATTATCAGTAA	
Fili	21888148	GTGCAAAAGCACGCAAACAC	
cbc	13484842	GTCGTCGGTCCCCATGGACGT	
CG17716	13646603	GTGGATGGGTGCAAGCGAGG	3
Cpr50Ca	13770617	GTAAAACAAAAATCATTATG	
Tango7	14164663	TTTGAGAGCATCGCTATTAG	
GEFmeso	18566638	TAATCTCGCGAAAAAGTGCA	
Sik3	18694657	TCCTCTAGTGAAAATCTGCG	4
5-HT1A	19078659	ATAAATTTATTTAACCCCTT	
Intergenic_9	20359591	ATCTAACTTACCGCTAAATG	
Intergenic_10	20754192	TGTAATGCTTCTTGTTTACA	
Intergenic_11	20755775	TTTCTCTCATTTACATTCAG	5
Intergenic_12	20804725	AAATAATTGGTCTGAACCAA	
Intergenic_13	20815303	AATTATTTAGTTGCATGTAT	
insc/sktl	20833839	CACCTTGTGTCCAATTTGTG	
cv-c	21377388	GTATTCAAAGTACGAAACGA	
Fkbp14	21495019	GGCAGAATGTCAGAGAGTTC	6
MESK2/CG10494	21516867	TTTGGGATTAAGTTAACAGT	

Polycistronic constructs used to creating pCFD5 cloning vector

- *D. melanogaster* tRNAgly (yellow)
- Rice tRNAgly (grey)
- Target gRNAs (green)
- gRNA core (cyan)

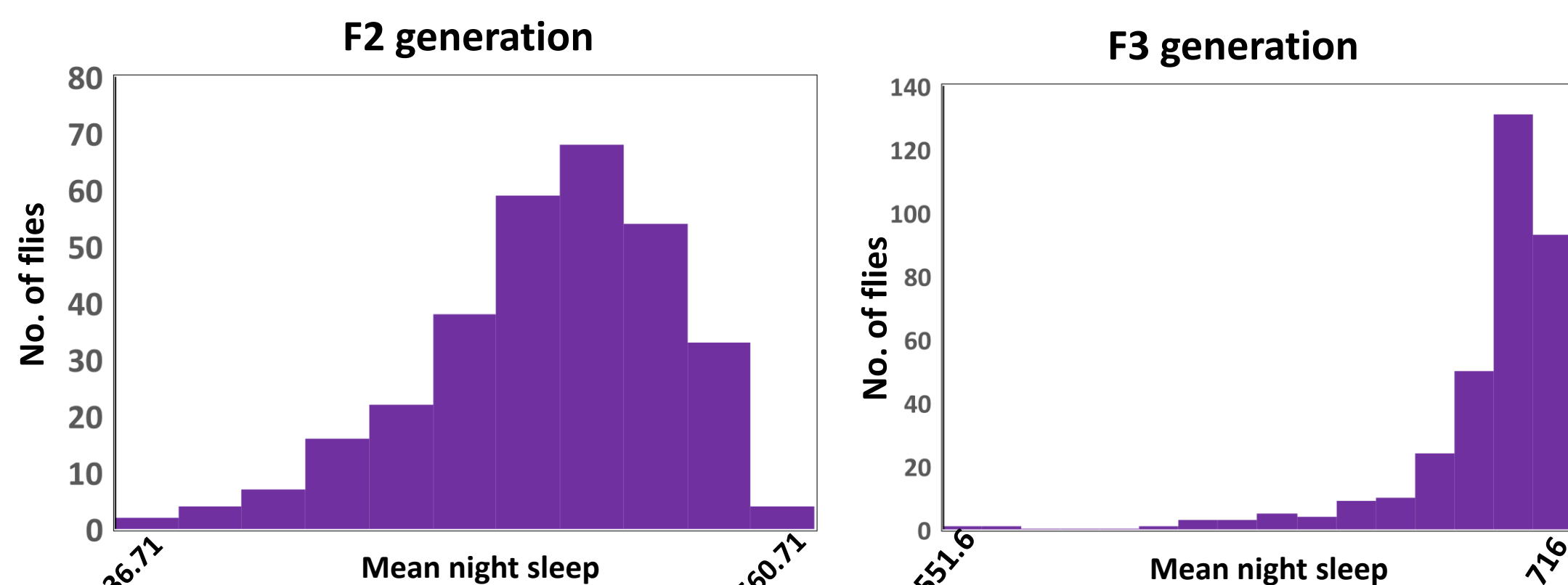### Schematic representation of extreme QTL mapping procedure

A long-sleeping line of the SIP, SIP_L1_9, was used for injection of pCFD5 plasmid containing each polycistronic construct. The average night sleep of SIP_L1_9 flies is 697 min and average day sleep is 492 min

1. Construct injected into SIP_L1_9 embryos — Injected parental flies
2. Injected flies allowed to mate randomly
3. F1 flies allowed to mate randomly to produce F2 — F1
4. F2 flies allowed to mate randomly to produce F3 — F2
5. Measure sleep in 400 F2 males and females. Freeze 45 flies of each sex with the longest and shortest sleep times.
6. Measure sleep in 400 F3 males and females. Freeze 45 flies of each sex with the longest and shortest sleep times. — F3
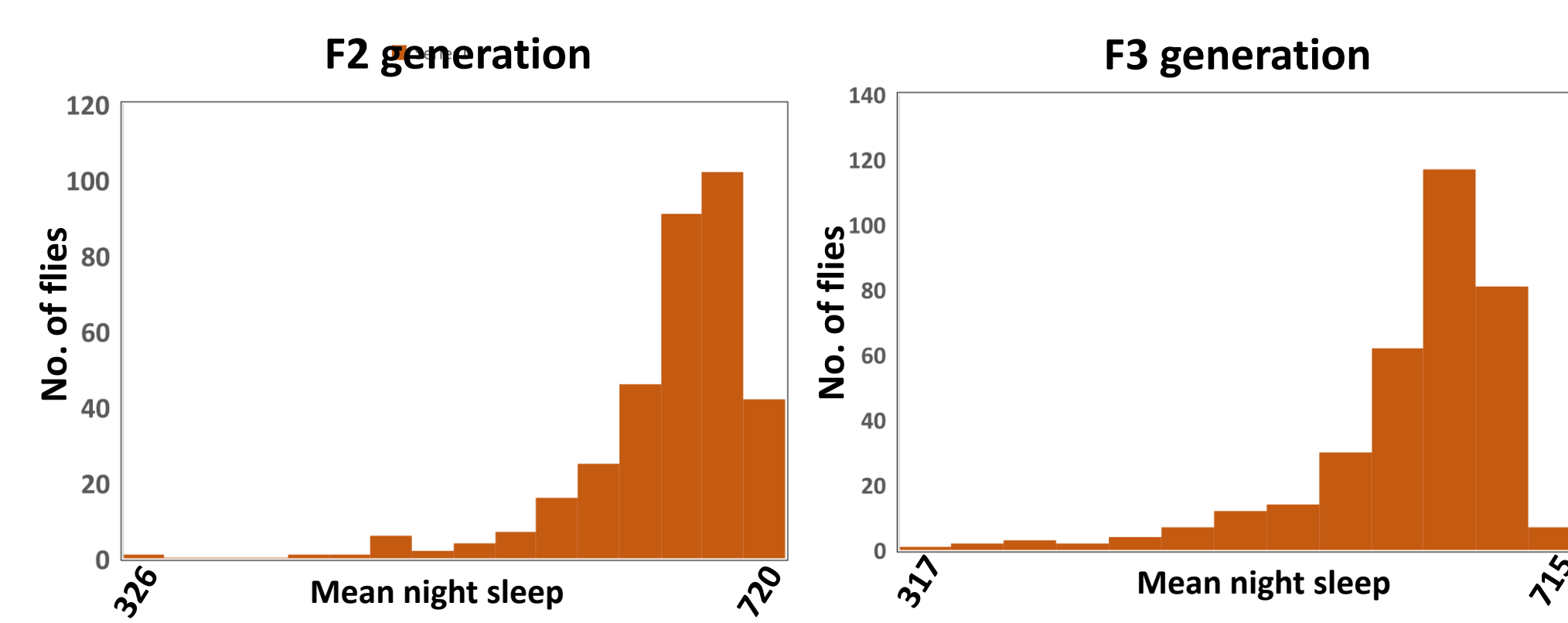
Sleep was measured for seven days using Drosophila Activity Monitors (DAM2; Trikinetics, Waltham, MA). We used a C# program (R. Sean Barnes) to calculate sleep parameters. We used SAS (SAS, Cary, NC) for statistical analysis.
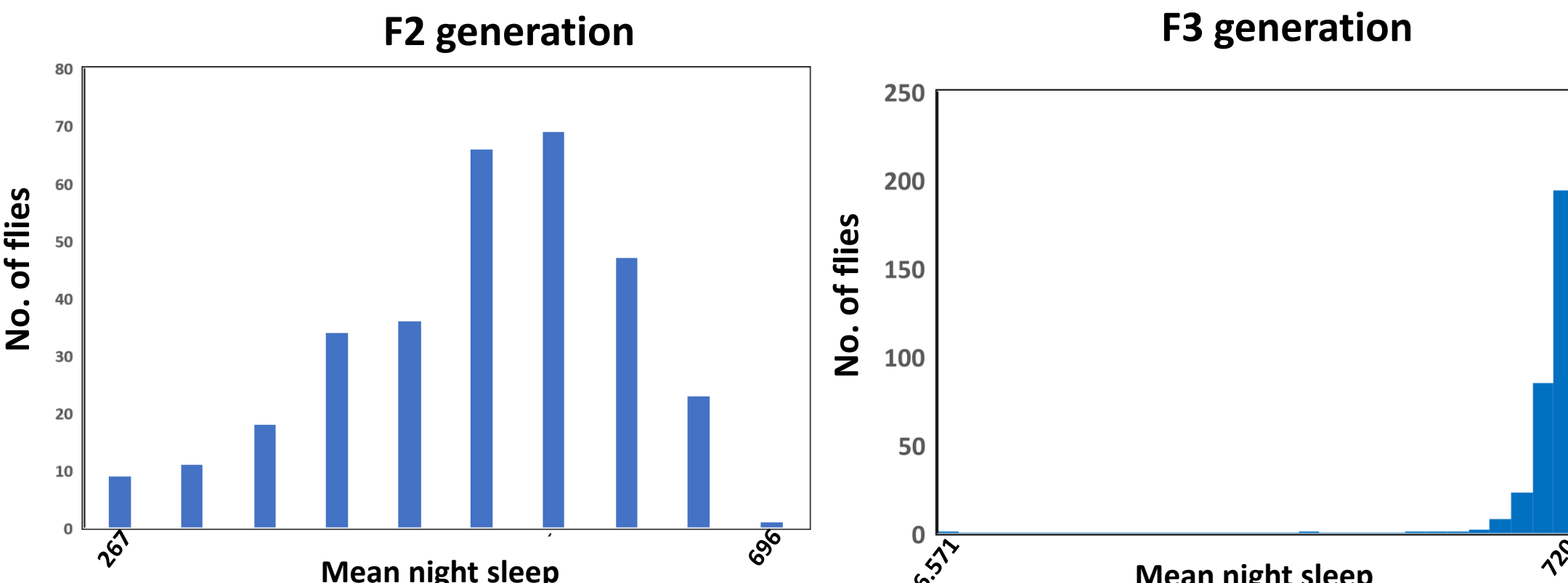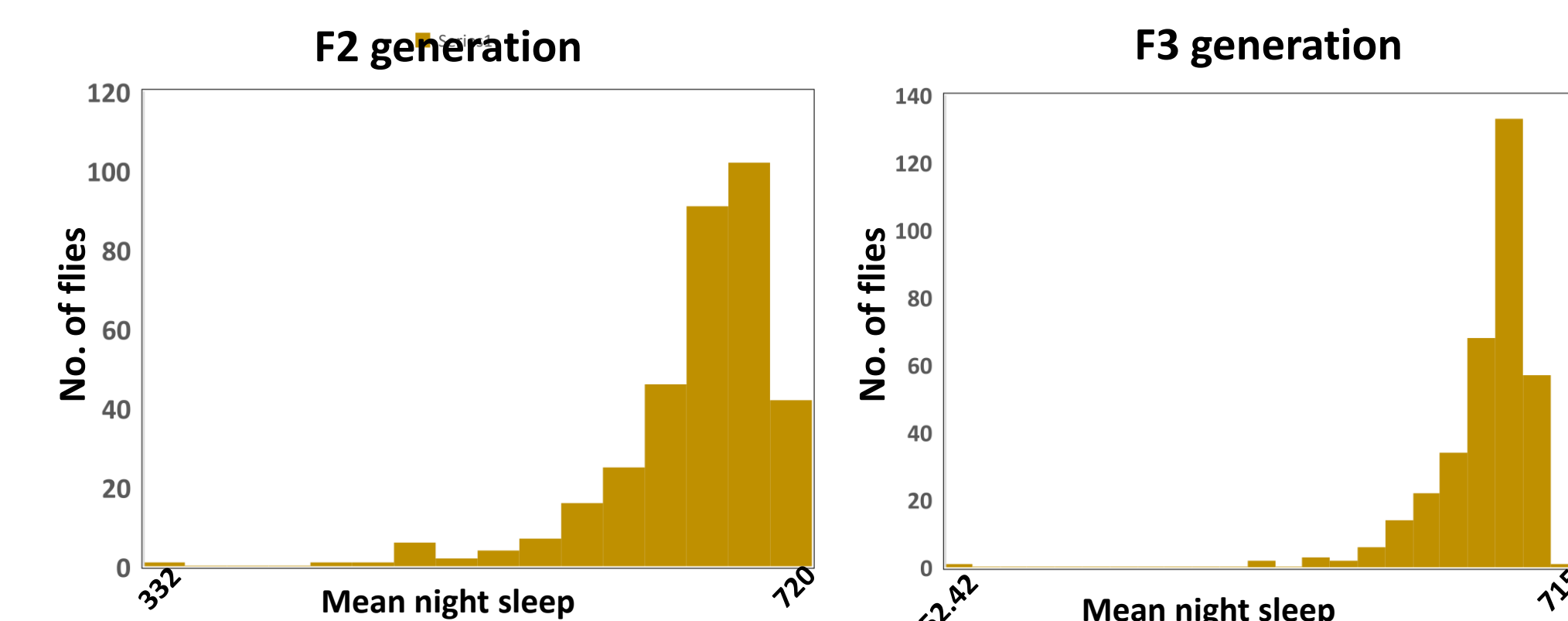
Results

Comparison of night sleep of transformed recombinants in F2/F3 generations injected with Construct 1

Comparison of night sleep of transformed recombinants in F2/F3 generations injected with Construct 2

Comparison of night sleep of transformed recombinants in F2/F3 generations injected with Construct 3

Comparison of night sleep of transformed recombinants in F2/F3 generations injected with Construct 4

Conclusions

- The distribution of night sleep shifted in flies injected with Constructs 1-4. In each case, the shift in the distribution was towards shorter sleep.
- For each distribution, the 10% shortest sleepers had significantly less sleep than the 10% longest sleepers (all *P* <0.0001), suggesting that the differences in sleep may map to these polymorphic variants.
- Sleep distributions in the F3 generation were narrower, suggesting that either 1) the double-stranded breaks were rare, or 2) that homozygous double-stranded breaks are lethal.
- Association of these phenotypes with the collected DNA will reveal the relationship between sleep and these variants.