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Developmental sleep reallocation enables metabolic adaptation in desert flies

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eLife Assessment

Li et al. present an **important** and innovative study linking developmental changes in sleep to ecological context in *Drosophila mojavensis*, and propose that sleep at one stage of an animal's life might anticipate needs at a future stage. The results fit well with this model, but are correlative in nature. The work is **convincing**, scientifically rigorous, and effectively bridges sleep biology and evolutionary ecology, opening promising new directions for the field.

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Abstract

Sleep is essential for adaptation and survival across the lifespan, yet the ecological pressures shaping sleep ontogeny remain poorly understood. We investigated sleep across early developmental stages in *Drosophila mojavensis*, a stress-resilient desert-adapted species. While adult *D. mojavensis* exhibit prolonged and consolidated sleep, along with enhanced starvation tolerance and survival compared to *Drosophila melanogaster*, the developmental trajectory underlying these adaptation strategies for surviving in harsh environments is unknown. Moreover, during developmental (larval) periods, animals do not encounter the same environmental stressors experienced by adults (e.g., food scarcity, extreme temperatures). We find that in contrast to adults, *D. mojavensis* larvae exhibit reduced and fragmented sleep relative to *D. melanogaster*. *D. mojavensis* larval sleep is also deeper, reflecting a shift toward increased sleep efficiency rather than simple sleep loss. *D. mojavensis* larvae consume more food than *D. melanogaster* and survive longer under starvation, suggesting a strategic tradeoff by suppressing sleep to prioritize nutrient intake and energy storage early in life while resources are more abundant. Metabolic analyses reveal elevated triglyceride accumulation in *D. mojavensis* across their lifespan, indicating enhanced energy storage capacity. These findings provide an example of how, within a fixed genetic background, an animal can reallocate sleep in opposing manners to maximize survival and energetics depending upon ecological pressures unique to each phase of life.

Introduction

Sleep is an evolutionarily conserved behavior essential for diverse physiological functions, yet natural sleep duration varies enormously across species ([1]; [2]). These interspecific differences often reflect ecological pressures. Under resource limitations, a fundamental trade-off arises: time spent asleep cannot be spent foraging, making sleep and feeding mutually exclusive behaviors. Many insects and mammals forgo sleep when faced with acute starvation to search for food,

underscoring a core conflict between sleep and feeding drives. Consistent with this, satiated animals tend to become quiescent post-feeding, whereas hunger strongly disrupts sleep ([3]). Thus, dynamic regulation of sleep and feeding in response to internal state and environmental conditions is thought to maximize fitness. However, the precise strategies by which animals tune these behaviors, particularly under extreme ecological conditions, remain poorly understood.

Environmental and physiological stressors reshape sleep behavior. Thermal stress inputs, such as extreme heat, activate neural circuits that disrupt sleep by triggering high sleep latency and sleep fragmentation ([4]; [5]). Paradoxically, accumulating evidence suggests that sleep itself can improve survival under such harsh conditions by conserving energy and maintaining homeostasis. Reduced locomotor activity and increased sleep duration have been shown to prolong survival during nutrient deprivation ([6]). A similar pattern has also been observed in the desert-adapted species *Drosophila mojavensis*: these flies maintain high sleep levels during food and water deprivation in contrast to *Drosophila melanogaster* and survive longer under such conditions, supporting the notion that elevated sleep fosters stress resilience and enhances resistance to resource scarcity ([7]). Related, in the nematode *Caenorhabditis elegans*, stress-induced sleep emerges after noxious stress exposure, and sleep-active neurons are essential for mediating sleep's protective role for survival ([8]; [9]). Taken together, sleep functions as a conserved adaptive response for enhancing stress tolerance.

Nearly all studies of sleep and stress have focused on adulthood. However, developmental sleep is hypothesized to fulfill physiological functions that differ from those in maturity ([10]). Across phylogeny, juveniles consistently sleep more than adults, and this ontogenetic transition in sleep timing is accompanied by functional changes to support stage-specific developmental processes. *Drosophila* larvae exhibit prolonged quiescent periods driven by specific neuromodulatory and neuropeptidergic circuits, facilitating developmental-specific functions such as neurogenesis and neural circuit assembly ([11]; [12]). Similar sleep patterns and functions have been observed in mammals across the lifespan, where neonates spend a greater proportion of time in REM sleep, which gradually declines with age ([15]; [16]). Sleep is thus not a static behavior but a highly plastic state that is restructured throughout development to meet dynamically changing physiological demands. Despite this, it remains unclear how the genetic and neurobiological adaptations that promote sleep-based resilience in adults shape sleep strategies earlier in life. Experimental evolution studies in *D.melanogaster* demonstrate that selection for adult starvation resistance increases adult sleep and reduces adult feeding, while producing developmentally specified changes such as extended development, elevated larval feeding, with little effect on larval sleep, implying stage-specific regulation of these traits ([17]). Here, we use *D.mojavensis* to test whether developmental sleep is reorganized within a genetic background optimized for adult survival, and whether it is tuned in anticipation of extreme conditions later in life.

Results

Sleep is reduced in *Drosophila mojavensis* larvae

Desert-adapted flies exhibit increased sleep in adulthood as part of a survival strategy under harsh conditions. However, during earlier developmental larval stages, these same animals do not encounter the same degree of food scarcity or temperature stress ([18]; [19]). To investigate whether physiological and genetic adaptations in desert flies promote fixed behavioral strategies across the lifespan, we initially assessed *D.mojavensis* second instar larval (L2) sleep under various rearing and experimental temperatures (Figure S1A [\[20\]](#)). We compared sleep architecture in three geographically segregated *D.mojavensis* subspecies (*Baja*, *Wrigleyi*, and *Mojavensis*) to that of wild-type strain *D.melanogaster* (*Canton-S*). When both species were reared and assayed at 25°C, *D.mojavensis* L2 slept substantially less than CS with similar locomotor activity, reflecting a reduced sleep phenotype not attributable to health or motor issues. *D.mojavensis* L2 sleep was also highly fragmented, characterized by markedly shorter sleep bouts (Figure 1A-D [\[21\]](#)). We noticed in these experiments that at 25°C, the standard lab temperature for *D.melanogaster*, *D.mojavensis* exhibited delayed development, failing to align with CS. To determine the optimal rearing conditions across species, we measured the time (hours) required for the embryo to reach the L2

stage. We found that raising *D.mojavensis* rearing temperature to 29°C synchronized *Baja*'s developmental progression with the control (Figure S1B). To examine whether the reduced sleep phenotype observed in *D.mojavensis* L2 stemmed from species-specific thermal preferences, we reared each species at its optimal developmental temperature and then assessed L2 sleep at 25°C or 29°C. This experimental design allowed us to dissociate developmental and acute temperature shift effects. Under both conditions, *D.mojavensis* L2 consistently showed a reduction in total sleep and shorter sleep bouts than *D.melanogaster*, indicating that the short, fragmented sleep phenotype persists regardless of temperature (Figure 1E-L [↗](#)). Thus, desert-adapted fly strains sleep less during larval stages, in contrast to excess sleep observed in adulthood.

To investigate whether dietary variances might have an impact on sleep, we assessed sleep in L2 raised on an *Opuntia* cactus-based medium, the natural host substrate of *D.mojavensis*, instead of standard agar-yeast food. We quantified sleep architecture in both the first (F1) and fifth (F5) generations after transitioning to the cactus diet to minimize potential short-term epigenetic effects resulting from laboratory exposures (Figure S2 [↗](#)). In both generations, *D.mojavensis* L2 exhibited reduced total sleep and fragmented sleep structure relative to *D.melanogaster*, indicating that the short-sleep phenotype is retained even on the natural diet. In addition to *D.mojavensis*, comparable sleep phenotypes were also observed in *D.arizonae*, a closely related desert species in the repleta group that shares similar ecological niches. The similarity in sleep traits points to a conserved short-sleep profile for larvae across desert-adapted *Drosophila* lineages (Figure S3 [↗](#)).

Intact sleep homeostasis and increased sleep efficiency

D.mojavensis larvae

To determine whether *D.mojavensis* L2's reduced, fragmented sleep reflects impaired homeostatic balance, we tested sleep rebound following sleep deprivation (SD). A high-intensity blue light pulse was intermittently delivered over a 3 hour window to disrupt sleep in *D.melanogaster* and *D.mojavensis* (Figure 2A [↗](#)). During the subsequent 3-hour recovery period, both sleep-deprived L2 species exhibited rebound sleep compared to non-deprived controls. In *D.melanogaster*, rebound was driven by upward trends in both sleep bout number and bout length; in *D.mojavensis*, rebound was driven by extension in sleep bout length (Figure 2B-C [↗](#)). As expected, waking activity was elevated during deprivation in both species, but during recovery it dipped slightly below baseline in *melanogaster*, consistent with compensatory suppression of activity to facilitate rebound sleep, whereas in *mojavensis* it returned to baseline levels (Figure 2D [↗](#)). The normal activity level after deprivation in *D.mojavensis* supports that observed differences in sleep parameters were not due to poor health. These results indicate that larval *D.mojavensis* have a normal homeostatic sleep response, analogous to that of *D.melanogaster* ([\[11\]](#)).

We next tested whether *D.mojavensis* L2 compensate for reduced sleep quantity by enhancing sleep quality. We assessed sleep depth via arousal-threshold experiments, delivering 4-second light stimuli of low (1 Amp) and high (5 Amp) intensity every 2 minutes over a 2-hour window. We observed that *D.mojavensis* L2 were less likely to awaken than *D.melanogaster* at both stimulus intensities (Figure 2E [↗](#), F), indicating a deeper sleep state. Activity levels both at baseline and in response the stimuli were similar between species, supporting that differences in arousal were sleep-specific rather than driven by disparities in light sensitivity (Figure 2E [↗](#), F).

Feeding is a compensatory behavior for sleep across the lifespan in *D.mojavensis*

Given *D.mojavensis* L2's limited sleep yet intact sleep need, we next asked whether their extra wake time is redirected toward an alternative adaptive behavior, specifically feeding. We hypothesized that *D.mojavensis* larvae may sacrifice sleep to increase nutrient intake and build energy reserves critical for long-term survival in their variable desert environment as adults. Using a dye-based absorbance feeding assay, we first quantified food consumption in L2. When a population of equal numbers of larvae were assessed, *D.mojavensis* groups consumed significantly

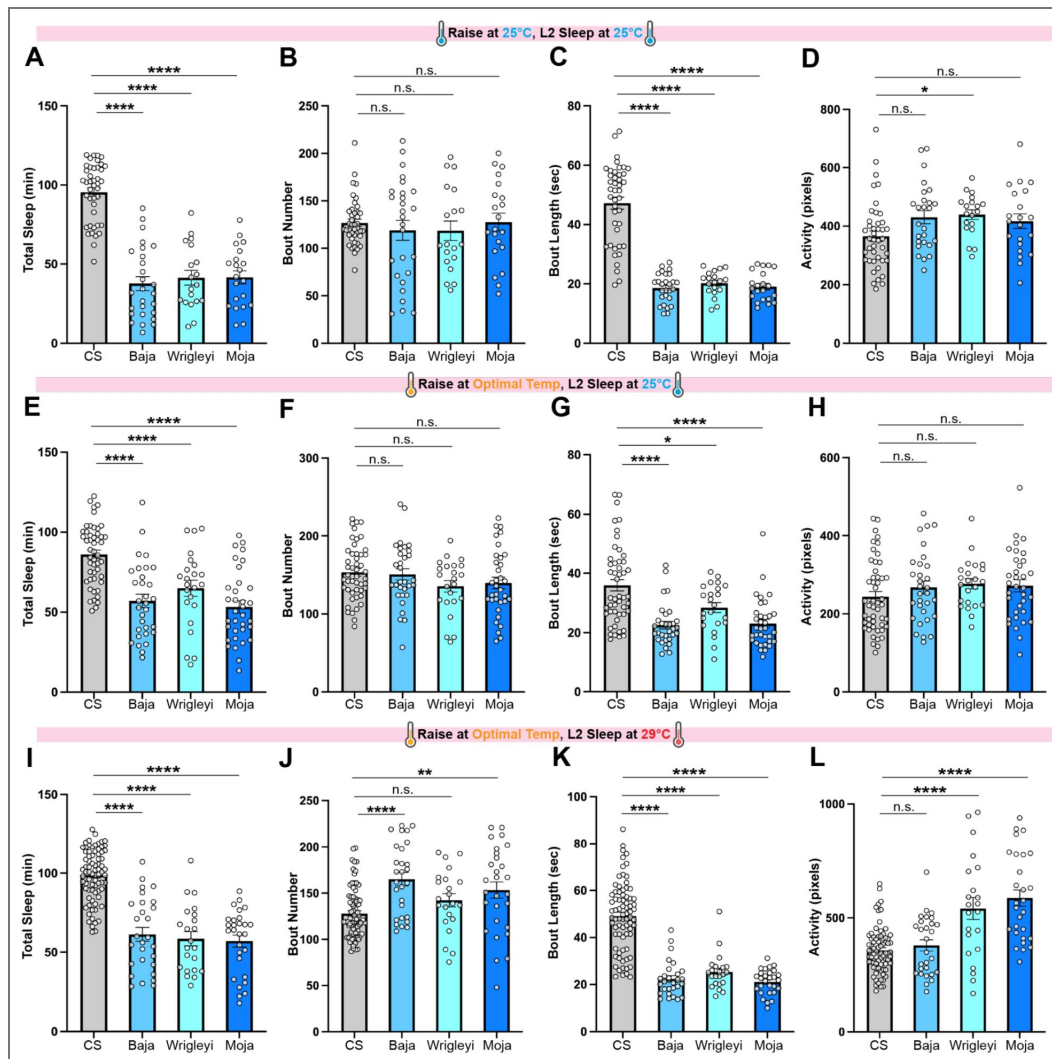


Figure 1. Reduced and fragmented sleep in *D.mojavensis* L2 across temperature conditions

(A-D) *D.melanogaster* (CS, grey, n=42) and *D.mojavensis* (*Baja*, light blue, n=26; *Wrigleyi*, cyan, n=19; *Moja*, dark blue, n=21) were reared at a uniform 25°C, and L2 sleep was measured over a three-hour interval at 25°C. (A) Total sleep duration. (B) Sleep bout number. (C) Sleep bout length. (D) Waking activity. (A-D) One-way ANOVAs followed by Dunnett's multiple comparisons test (**** p<0.0001, * p<0.05, n.s. p>0.05). (E-H) *D.melanogaster* (CS, grey, n=49) and *D.mojavensis* (*Baja*, light blue, n=31; *Wrigleyi*, cyan, n=23; *Moja*, dark blue, n=34) were raised at their optimal temperatures (*D.melanogaster*, 25°C; *D.mojavensis*, 29°C), and L2 sleep was measured over a three-hour interval at 25°C. (E) Total sleep duration. (F) Sleep bout number. (G) Sleep bout length. (H) Waking activity. (E-H) One-way ANOVAs followed by Dunnett's multiple comparisons test (**** p<0.0001, * p<0.05, n.s. p>0.05). (I-L) *D.melanogaster* (CS, grey, n=77) and *D.mojavensis* (*Baja*, light blue, n=28; *Wrigleyi*, cyan, n=22; *Moja*, dark blue, n=28) were raised at their optimal temperatures, and L2 sleep was measured over a three-hour interval at 29°C. (I) Total sleep duration. (J) Sleep bout number. (K) Sleep bout length. (L) Waking activity. (I-L) One-way ANOVAs followed by Dunnett's multiple comparisons test (**** p<0.0001, ** p<0.01, * p<0.05, n.s. p>0.05).

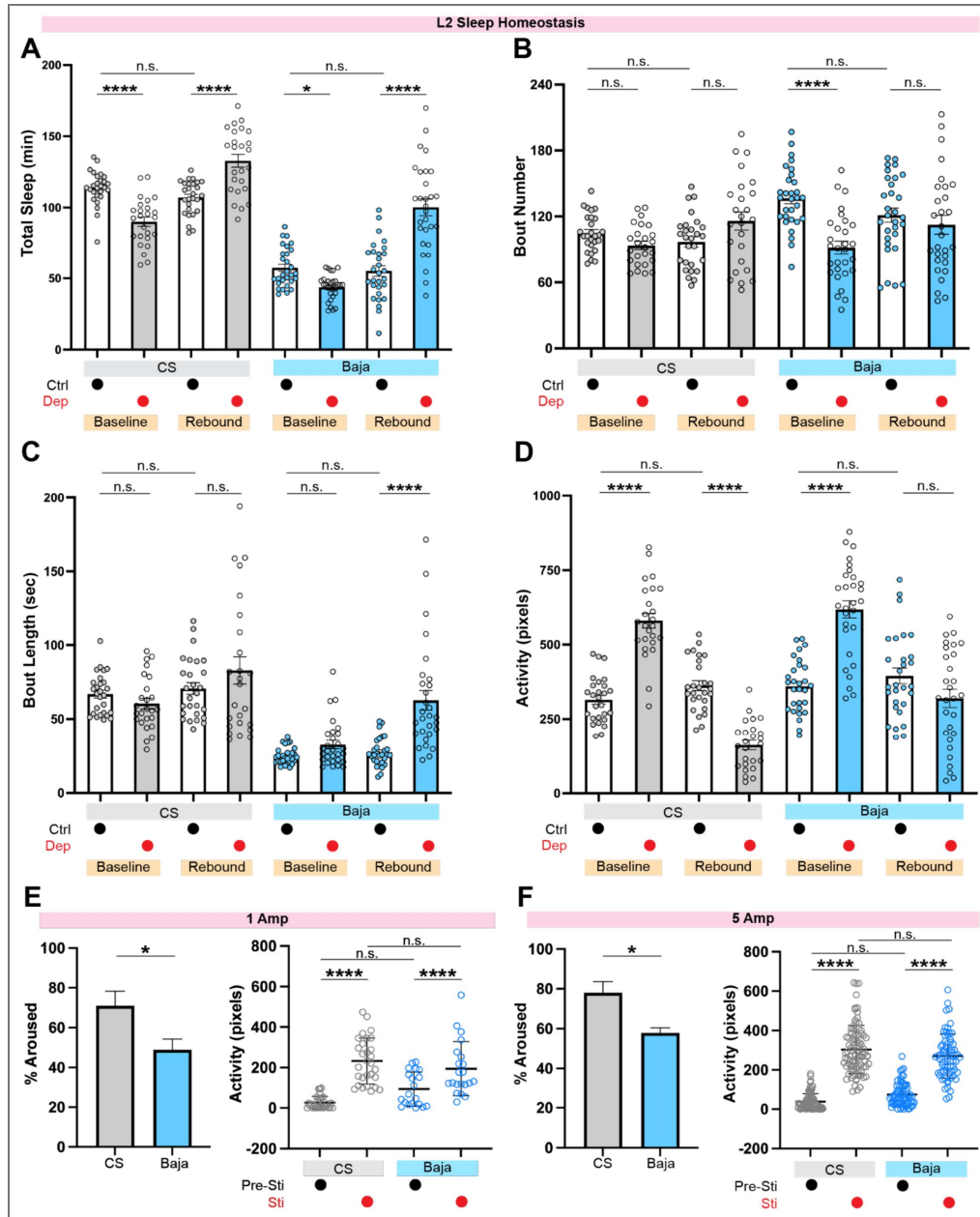


Figure 2. Homeostatic sleep regulation and higher arousal threshold in *D.mojavensis* L2

(A–D) *D.melanogaster* (CS, grey) and *D.mojavensis* (Baja, light blue) L2 were subjected to 3 hours of sleep deprivation (SD) via repetitive light pulses (Baseline) followed by a 3-hour recovery period (Rebound) (CS Baseline Ctrl, n=27; CS Baseline Dep, n=25; CS Rebound Ctrl, n=27; CS Rebound Dep, n=25; Baja Baseline Ctrl, n=30; Baja Baseline Dep, n=29; Baja Rebound Ctrl, n=30; Baja Rebound Dep, n=29). Quantifications of (A) total sleep duration, (B) sleep bout number, (C) Sleep bout length, and (D) waking activity demonstrate intact sleep homeostasis in *D.mojavensis* L2. (A–D) One-way ANOVAs followed by Sidak's multiple comparisons test (**** p<0.0001, * p<0.05, n.s. p>0.05). (E) *D.melanogaster* (CS, grey, n=4 replicates) and *D.mojavensis* (Baja, light blue, n=5 replicates) L2 were exposed to repetitive low-intensity (1 Amp) light pulses over a 2-hour testing window. The percentage of L2 aroused upon stimulation was measured, and activity levels before (Pre-Sti) and during (Sti) stimulation were recorded to assess baseline light responsiveness (CS Pre-Sti, n=30; CS Sti, n=30; Baja Pre-Sti, n=21; Baja Sti, n=21). (E) Arousal threshold comparison: unpaired two-tailed Student's t-test (* p<0.05). Activity quantification: one-way ANOVAs followed by Sidak's multiple comparisons test (**** p<0.0001, n.s. p>0.05). (F) *D.melanogaster* (CS, grey, n=3 replicates) and *D.mojavensis* (Baja, light blue, n=3 replicates) L2 were exposed to repetitive high-intensity (5 Amp) light pulses over a 2-hour testing window. L2 arousal and activity were quantified as in (E) (CS Pre-Sti, n=88; CS Sti, n=88; Baja Pre-Sti, n=68; Baja Sti, n=68). (F) Arousal threshold comparison: unpaired two-tailed Student's t-test (* p<0.05). Activity quantification: one-way ANOVAs followed by Sidak's multiple comparisons test (**** p<0.0001, n.s. p>0.05).

more on average than *D.melanogaster* (Figure 3A [↗](#)), suggesting a shift in behavioral priority. To rule out the possibility of a body size confound, we measured weight across the life cycle and found that *D.mojavensis* L2 are half the body weight of *D.melanogaster* at this stage (Figure 3B [↗](#)), suggesting an underestimation of feeding disparities. To account for this scaling difference, we repeated the feeding assay under a weight-matched design by doubling the sample size of *D.mojavensis* L2 so that the total biomass matched the *D.melanogaster* group. The increased feeding indeed appeared even greater with this design (Figure 3C [↗](#)), confirming enhanced nutrient intake in *D.mojavensis* L2. We then asked whether this sleep-feeding tradeoff is limited to the L2 stage or maintained across larval development. In third instar larvae (L3), *D.mojavensis* likewise exhibited reduced and fragmented sleep compared to *D.melanogaster* (Figure 3D-G [↗](#)). These altered sleep parameters were again accompanied by an increase in food consumption (Figure 3H [↗](#)).

In stark contrast to larval stages, *D.mojavensis* adults exhibit elevated and consolidated sleep compared to *D.melanogaster*. We thus investigated the sleep-feeding relationship at this stage using a liquid food interaction assay and found adult *D.mojavensis* engaged in significantly fewer feeding interactions, with reduced licking frequency, shorter feeding durations, and fewer feeding events compared to *D.melanogaster* adults (Figure 3I-K [↗](#)) ([20]). Together, these results suggest that *D.mojavensis* larvae may actively extend their wake time to engage in foraging and feeding, thereby conserving and storing energy early in development when metabolic demands are high, resources are abundant, and threats are minimal. In adulthood, scarce resources and unpredictable survival conditions shift *D.mojavensis* from a feeding-dominant to a sleep-dominant strategy, prioritizing energy conservation. To further explore the physiological basis of this shift, we measured triglycerides (TAG) energy stores across development. *D.mojavensis* accumulated more lipid reserves than *D.melanogaster* by the L3 stage and sustained into adulthood (Figure 3L [↗](#)). These findings reveal a life-stage-dependent reorganization of behavioral priorities in *D.mojavensis*, aligning sleep-feeding strategies with dynamically changing survival demands.

Differential starvation responses and enhanced survival in *D.mojavensis* L2

We next directly tested the functional consequences of *D.mojavensis*'s sleep and metabolic profile under acute food stress. In a food-deprived starvation paradigm, both *D.mojavensis* and *D.melanogaster* L2 showed the expected response of suppressing sleep relative to fed controls, consistent with hunger-induced foraging behavior. However, closer examination revealed that *D.mojavensis* L2 suppressed sleep to a lesser extent than *D.melanogaster* under starvation, including greater sleep duration, more sleep bouts, and longer bout length (Figure 4A-C [↗](#)). Thus, while both species suppress sleep in the absence of food, *D.mojavensis* L2 do not curtail sleep as drastically as *D.melanogaster*, perhaps reflecting shared adaptations with desert fly adults that promote starvation resistance.

How do starvation response behavioral differences affect survival? Adult *D.mojavensis* are able to survive longer than *D.melanogaster* in the absence of food and even water ([7]). Under starvation conditions, *D.mojavensis* L2 also survived longer than *D.melanogaster* L2 at both 25°C (Figure 4E [↗](#)) and 29°C (Figure 4F [↗](#)), indicating enhanced resilience under stress. Notably, although adult *D.mojavensis* are starvation resistant, they do not suppress sleep in the absence of food as adult *D.melanogaster* do. This finding reinforces the concept of distinct, developmental stage-specific survival strategies in *D.mojavensis*. Larvae actively suppress sleep to maximize foraging opportunities during starvation, whereas adults apparently adopt a different approach, possibly conserving energy by maintaining sleep and relying on pre-accumulated energy reserves (consistent with the elevated larval and adult TAG levels) rather than active foraging.

Discussion

Here we report that in contrast to adulthood, the desert-adapted *D.mojavensis* reallocate time from sleep to feeding during developmental larval stages. These larvae exhibit reduced and fragmented sleep alongside markedly elevated feeding, a behavioral shift that correlates with enhanced

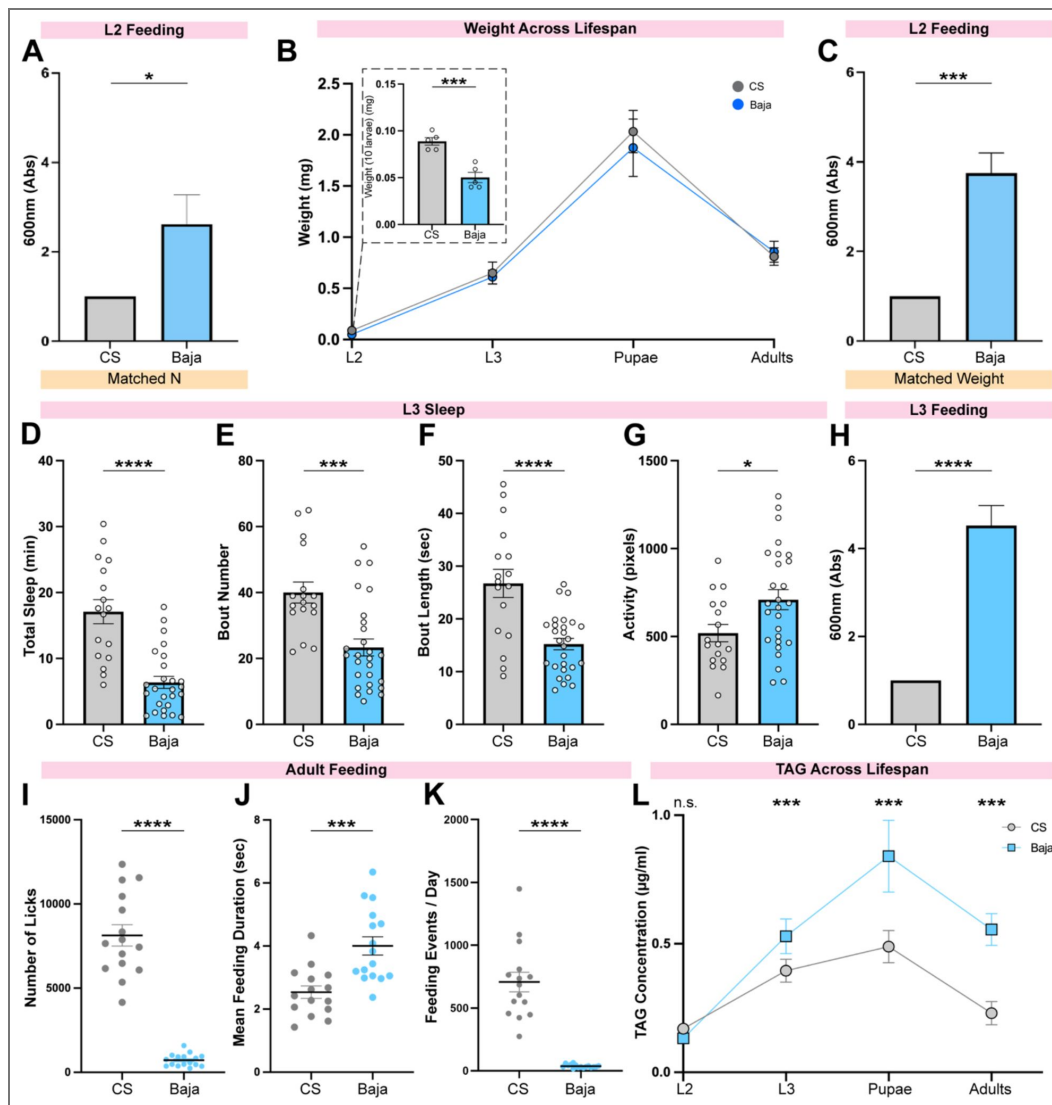


Figure 3. Enhanced larval feeding and developmental energy storage

(A-C) *D. melanogaster* (CS, grey) and *D. mojavensis* (Baja, light blue) L2 were assayed for food intake using a dye-based absorbance assay. (A) Under matched sample sizes (Matched N) (CS, n=5 replicates; Baja, n=5 replicates). Unpaired two-tailed Student's t-test (* $p < 0.05$). (B) Body weight measurements across life stages (CS, n=5 replicates for each stage; Baja, n=5 replicates for each stage). Unpaired two-tailed Student's t-test (*** $p < 0.001$). (C) Under weight-matched conditions (Matched Weight) (CS, n=5 replicates; Baja, n=5 replicates). Unpaired two-tailed Student's t-test (*** $p < 0.001$). (D-G) *D. melanogaster* (CS, grey, n=17) and *D. mojavensis* (Baja, light blue, n=27) L3 were analyzed for sleep. (D) Total sleep duration. (E) Sleep bout number. (F) Sleep bout length. (G) Waking activity. (D-G) Unpaired two-tailed Student's t-test (**** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$). (H) *D. melanogaster* (CS, grey, n=5 replicates) and *D. mojavensis* (Baja, light blue, n=5 replicates) L3 were analyzed for feeding. Unpaired two-tailed Student's t-test (**** $p < 0.0001$). (I-K) *D. melanogaster* (CS, grey) and *D. mojavensis* (Baja, light blue) adults were analyzed for feeding. (I) The total number of individual food interactions over 24h was significantly elevated in adult CS females relative to Baja. Welch's t-test $t = 11.57$, $p < 0.0001$. (J) The mean duration of feeding events that include consecutive licks was prolonged in Baja adults compared to CS. Welch's t-test $t = 4.164$, $p = 0.0003$. (K) The total number of feeding events per day was increased in CS adult females relative to Baja. Welch's t-test $t = 8.608$, $p < 0.0001$. n=15-16 flies/group for (I-K). (L) Whole-body triglyceride (TAG) concentrations across development (L2, n=25/group; L3, n=25/group; pupae, n=5/group; adults, n=5/group). TAG levels were comparable at the L2 stage but became significantly higher in *D. mojavensis* from L3 onward, indicating increased energy storage initiated in later larval stages and sustained into adulthood. Unpaired two-tailed Student's t-test (*** $p < 0.001$).

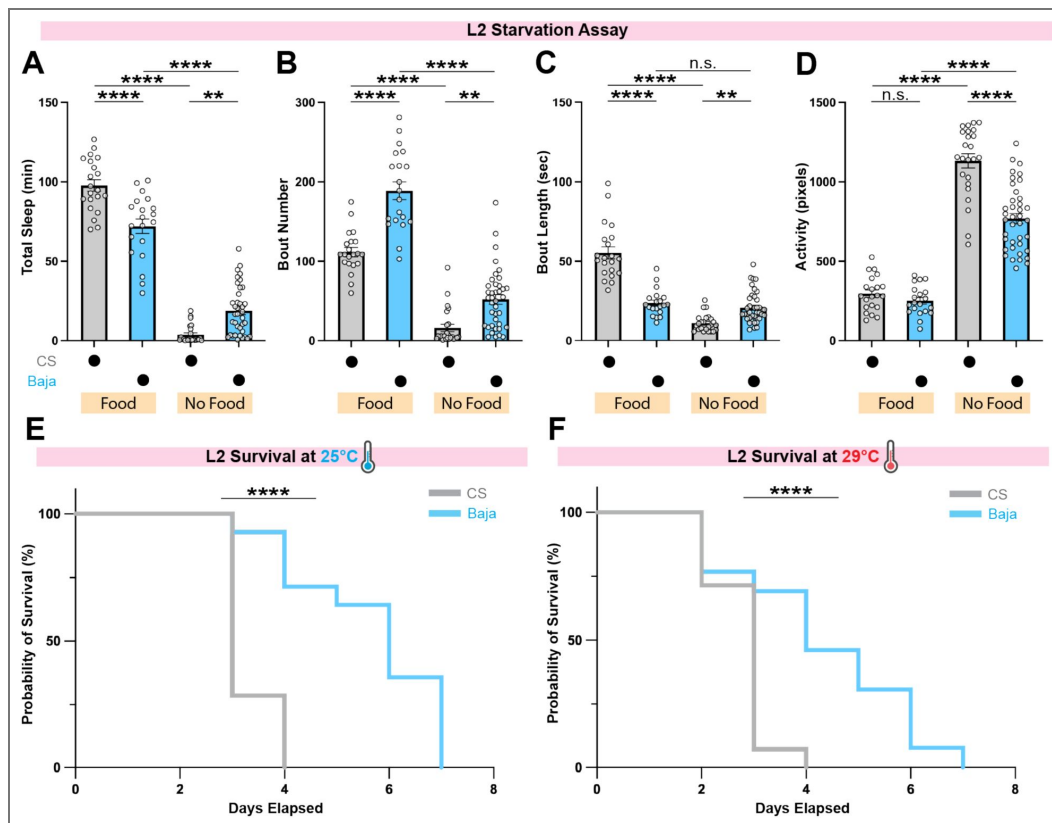


Figure 4. *D.mojavensis* L2 exhibit differential behavioral responses to starvation and enhanced survival.

(A-D) *D.melanogaster* (CS, grey, n=21 for “Food”, and n=24 for “No Food”) and *D.mojavensis* (*Baja*, light blue, n=20 for “Food”, and n=39 for “No Food”) L3 were analyzed for sleep under starvation stresses. (A) Total sleep duration. (B) Sleep bout number. (C) Sleep bout length. (D) Waking activity. (A-D) One-way ANOVAs followed by Tukey’s multiple comparisons test (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$). (E-F) Survival curves of *D.melanogaster* (CS, grey) and *D.mojavensis* (*Baja*, light blue) L2 larvae under starvation conditions. (E) Probability of survival at 25°C. *D.mojavensis* L2 (*Baja*, n=14) show significantly longer survival compared to *D.melanogaster* L2 (CS, n=14) under starvation. (F) Probability of survival at 25°C. *D.mojavensis* L2 (*Baja*, n=14) show significantly longer survival compared to *D.melanogaster* L2 (CS, n=14) under starvation. (E-F) Simple survival analysis (Kaplan-Meier) followed by Log-rank (Mantel-Cox) test (**** $p < 0.0001$).

starvation resilience. Adult *D.mojavensis* not only show excessive sleep but also feed less. Thus, the short-sleeping, food-seeking larvae and the long-sleeping adults represent dissociated phenotypes within the same species. This dissociation between life stages underscores the evolutionary importance of stage-specific energy budgeting. In a desert environment where necrotic cactus substrates are patchy and unpredictable, selection likely favored larvae that suppress sleep to exploit transient food resources and accumulate critical metabolic reserves for later development. The adults, no longer constrained by growth or feeding, can afford to sleep longer as a water- and energy-conserving strategy. This developmental compartmentalization highlights the ecological significance of plastic sleep architecture, with evolution tuning behavioral priorities across ontogeny in response to fluctuating environmental pressures ([15];[20]). Consistent with this ecological framework, our starvation assays provide empirical validation for the adaptive advantage conferred by the larval phenotypes observed in *D.mojavensis*. The ability to survive for more extended periods without food directly supports the hypothesis that reduced sleep, increased feeding, and heightened arousal threshold are integral components of a robust strategy for resilience in unpredictable environments. The suite of behavioral and metabolic adaptations in larvae (devoting wakefulness for foraging, efficiently utilizing resources, and moderating sleep loss under starvation) directly contributes to their ability to withstand periods of food scarcity.

The persistence of elevated arousal threshold (deeper sleep profile) across life stages suggests a conserved shift toward higher sleep efficiency ([7]). Though the absolute sleep need in larvae versus adults might differ, sleep remains essential for *D.mojavensis*, even if the underlying physiological functions differ between development and adulthood. At a mechanistic level, we propose that the short and fragmented sleep in *D.mojavensis* larvae is a genetically encoded adaptation that initiates, rather than responds to, downstream metabolic and developmental consequences. Despite a shared genome between larva and adult stages, larvae's distinct sleep phenotype suggests that the genes regulating sleep are deployed in a stage-specific manner, likely shaped by developmental context and ecological necessity. If adult sleep elevation were driven by mutations in broadly acting sleep-promoting pathways, we might expect larval sleep to be similarly increased. The reduced and fragmented larval sleep instead implies either that larval and adult sleep are regulated by distinct genetic programs, or that *D.mojavensis* has evolved independent genetic control across development. This interpretation aligns with previous work showing that selection for adult sleep or starvation resistance in *D.melanogaster* does not produce parallel changes in larval behavior ([6]; [17]), supporting the idea that sleep is modularly regulated across ontogeny. This framework of stage-specific sleep regulation is reinforced by neurobiological evidence. In *D.melanogaster*, neuromodulators governing sleep differs across development ([11]). This organization suggests that evolutionary changes can adjust sleep at one developmental stage without necessarily disrupting sleep regulation or homeostasis at other stages. Evolutionary changes in neuromodulator expression and/or sleep-wake circuits across development could give rise to a short-sleeping larval phenotype that drives metabolic investment, and a long-sleeping adult phenotype that enhances stress resilience.

More broadly, our work contributes to a growing body of evidence that sleep is a flexible, ecologically shaped behavior. Across taxa, animals adjust sleep timing and duration to accommodate life-history events such as migration, breeding, and seasonal foraging. White-crowned sparrows reduce sleep dramatically during migration while maintaining cognitive performance, and Arctic sandpipers virtually eliminate sleep during mating season to maximize reproductive success ([21];[22]). In insects, male *Drosophila* suppress nighttime sleep to court females ([24];[25]). These examples show that animals can sacrifice sleep when ecological demands take priority. *D.mojavensis* larvae offer a developmental parallel as securing nutrients outweighs the need for sustained sleep at this stage. An important next step will be to identify the neurogenetic mechanisms that govern this reallocation. Given that neuromodulators such as dopamine, octopamine, and NPF are known to regulate sleep and feeding in adults, one hypothesis is that these systems are developmentally re-tuned in *D.mojavensis* larvae to permit prolonged wakefulness without impairing developmental progression ([26];[27]). Taken together, our findings reveal a developmentally reprogrammed, short-sleep phenotype in desert-adapted larvae,

driven by ecological pressures and aligned with survival demands. This work advances the view that sleep is not static but a highly plastic state, reorganized across life stages in response to environmental and physiological priorities.

Methods and Materials

Adult Rearing

Fly stocks were cultured on standard cornmeal molasses media (per 1L H₂O: 12 g agar, 29 g Red Star yeast, 71 g cornmeal, 92 g molasses, 16mL methyl paraben 10% in EtOH, 10mL propionic acid 50% in H₂O) at 25°C with 60% relative humidity and entrained to a daily 12h light, 12h dark schedule. *Canton-S* (CS) were provided by Dr. Gero Miesenböck (University of Oxford). Primary stocks *D.mojavensis.baja* (collected March 2020, La Paz, Mexico), *D. mojavensis.moj* (collected February 2020, North Joshua Tree National Park, CA), *D.mojavensis.wrigleyi* (collected November 2017, Catalina Island, CA) were a gift from Dr. Luciano Matzkin (University of Arizona). *D. arizonae* were ordered from the National *Drosophila* Species Stock Center (SKU: 15081-1271.36).

Larval Rearing

All fly stocks were cultured on a standard molasses medium and raised in incubators on a 12:12 light:dark (LD) cycle. Different optimal raising temperatures were used across species to synchronize their developmental time: CS was maintained at 25°C; *baja*, *moja*, *wrigleyi*, and *arizonae* at 29°C. To collect synchronized L2, adult flies were placed in an embryo collection cage (Genesee Scientific, cat#: 59-100) and eggs were laid on a petri dish containing 3% sugar, 2% sucrose, and 2.5% apple juice with yeast paste on top.

Larval Body Weight Measurements

For weight, groups of 10 L2, 5 L3, pupae, or adults were washed in Milli-Q water and dried using a Kimwipe. The samples were then weighed as a group on a scale, and the weight in milligrams (mg) was recorded.

Sleep Assays and Starvation Assays

On the day of the sleep assay, molting first instar larvae (L1) were collected and moved to a separate dish to complete the molt to the L2. Freshly molted L2 were placed into individual wells of LarvaLodge containing 100 µl of 3% agar and 2% sucrose media covered with a thin layer of yeast paste. Experiments with Banana-Opuntia media used a recipe from the National *Drosophila* Species Stock Center (NDSSC; Cornell University): per 1L H₂O: 14.16g agar, 27.5 g yeast, 2.23g methyl paraben, 137.5g blended bananas, 95g Karo Syrup, 30g Liquid Malt Extract, 22.33g 100% EtOH, 2.125g powdered opuntia cactus.

For starvation assays, 100 µl of 3% agar-only media was applied in each well. The LarvaLodge was covered with a transparent acrylic sheet brushed with a thin layer of detergent avoiding condensation, and moved into a DigiTherm incubator at either 25°C or 29°C for imaging in constant dark.

LarvaLodge Image Acquisition and Processing

Images were captured every 6 sec for 5 hrs with an Imaging Source DMK 23GP031 camera equipped with a Fujinon lens with a Hoya 49mm R72 Infrared Filter. We used IC Capture (The Imaging Source) to acquire time-lapse images. All experiments were carried out in the dark using infrared LED strips positioned below the LarvaLodge.

Images were analyzed using custom-written MATLAB software. Temporally adjacent images were subtracted to generate maps of pixel value intensity change. A binary threshold was set such that individual pixel intensity changes that fell below 40 gray-scale units within each well were set equal to zero (“no change”) to eliminate noise. Pixel changes greater than or equal to the threshold

value were set equal to one (“change”). Activity was then calculated by taking the sum of all pixels changed between images. Sleep was defined as an activity value of zero between frames. Total sleep was summed for 3 hrs beginning 1 hr after the molt to L2.

Arousal Threshold, Light Responsiveness, and Sleep Deprivation via Blue Light Stimulation

Blue light stimulation was delivered using two high-power LEDs (Luminus Phatlight PT-121, 460 nm peak wavelength, Sunnyvale, CA) secured to an aluminum heat sink and driven at a constant current of 1 Amp. The blue light stimulus was delivered for 4 sec every 2 min for 1 hr beginning the 2nd hr after the molt to L2. The number of larvae showing activity changes in response to the stimulus was counted, and the percentage of larvae that moved in response to the stimulus was recorded for each experiment.

For light responsiveness, we derived the arousal threshold data for further processing. We compared larval activity (pixel) 30 sec before each stimulus and 30 sec after each stimulus of all 30 light stimuli for individuals in the sleep state 6 sec before light stimulation and awake during the first light stimulation.

For sleep deprivation, we used a 5-Amp stimulus for 18 sec every 30 sec for 3 hrs beginning 1 hr after the molt to L2. Undisturbed control larvae were placed in a separate incubator under identical conditions without blue light exposure during the deprivation.

Feeding Behavior Analysis

Freshly molted L2 were placed in a petri dish containing blue-dyed 3% agar, 2% sucrose, and 2.5% apple juice with blue-dyed yeast paste on top for 4 hrs at 25°C in constant darkness. After 4 hrs, L2 were washed in water, transferred to microtubes, and frozen at -80°C overnight. 300 µl of distilled water and spun down for 5 min at 13,000 rpm. The amount of blue dye in the supernatant was then measured using a spectrophotometer (OD₆₂₉). Food intake represents the OD value of each measurement.

Triglyceride (TAG) and Bicinchoninic Acid Protein (BCA) Assays

Triglyceride levels were measured using the Stanbio Triglyceride LiquiColor Kit (EMSCO/Fisher, 2100-430) from *Drosophila* adult flies, pupae, and larvae. The sample sizes for adults, pupae, and larvae were n = 5, n = 5, and n = 25, respectively. Samples were collected and washed in cold phosphate-buffered saline (PBS) and homogenized 3 x 10 sec each in 125 µL of lysis buffer (140 mM NaCl, 50 mM Tris-HCl [pH 7.4], 20% Triton X-100, and 1X protease inhibitors). The homogenates were centrifuged at 13,000 rpm for 10 min at 4°C, and the supernatants were stored at -20°C until analysis. TAG levels (µg/µL) were recorded according to the kit protocol and measured using a microplate reader at 500 nm. TAG data were normalized to protein levels measured at 562 nm, quantified using the Pierce BCA Protein Assay Kit (Cell Center/Thermo, PI23227).

Survival with Starvation Analysis

Freshly molted L2 were placed into individual wells of LarvaLodge containing 100 µl of 3% agar, transferred into a DigiTherm incubator at either 25°C or 29°C, and imaged in constant dark for an infinite time until all L2 were observed and determined deceased. To prevent condensation, the transparent acrylic sheet covering the wells was replaced every 24 hours.

FLIC Feeding Assay

Fly Liquid Interaction Counter (FLIC; Sable Systems, North Las Vegas, NV) feeding assay was performed to obtain data on feeding duration and frequency previously described ([20]). 4-8 day old female flies were individually loaded into separate chambers of the *Drosophila* Feeding Monitors (DFM) to track feeding behavior. DFM feeding wells were loaded with a solution of 5.0% sucrose and 50 mg/L MgCl₂ in distilled water. Individual flies are aspirated into separate DFM

chambers between ZT0-2 and behavior data is recorded for 24h. Feeding data includes measurements from two independent experiments. The DFM detects feeding signals from individual flies via a microcontroller circuit board on each well. Raw feeding signal data is coordinated and processed in the Master Control Unit (MCU). Feeding data was analyzed in R Studio using previously described analysis scripts (https://github.com/PletcherLab/FLIC_R_Code) ([28]).

Statistical Analysis

All statistical analysis was done in GraphPad (Prism). For comparisons between 2 conditions, two-tailed unpaired *t*-tests were used. For comparisons between multiple groups, ordinary one-way ANOVAs followed by Tukey's multiple comparison tests were used. For comparisons between different groups in the same analysis, ordinary one-way ANOVAs followed by Sidak's multiple comparisons tests were used.

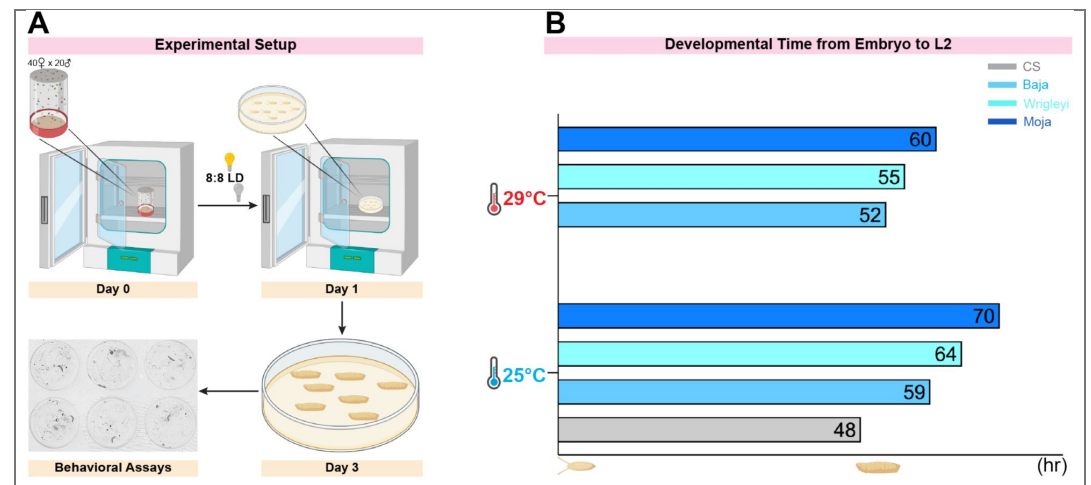


Figure S1. Experimental design schematic and developmental timing synchronization across species (A) Schematic of the experimental design. Embryos were collected from crosses of 40 ♀ female × 20 ♂ male flies and reared on agar-yeast plates under an 8:8 light-dark (LD) cycle. Larvae were aged to the second instar larval stage (L2) over three days, and freshly molted L2 were collected for subsequent behavioral assays. **(B)** Mean developmental time (hours from embryo to L2) for *D.melanogaster* (CS, grey, n=20) and three *D.mojavensis* subspecies (*Baja*, light blue, n=20; *Wrigleyi*, cyan, n=20; *Mojavensis*, dark blue, n=20) at 25°C and 29°C. Created with BioRender.com.

Figure S2. Reduced and fragmented sleep in *D.mojavensis* L2 persists on natural cactus-based food across generations.

(A-D) *D.melanogaster* (CS, grey, n=27) and *D.mojavensis* (Baja, light blue, n=26) P0 generation were raised on banana-Opuntia cactus medium. L2 sleep was measured over a 3-hour interval in the F1 generation. (A) Total sleep duration. (B) Sleep bout number. (C) Sleep bout length. (D) Waking activity. (A-D) Unpaired two-tailed Student's t-test (**** p<0.0001, n.s. p>0.05). (E-H) *D.melanogaster* (CS, grey, n=27) and *D.mojavensis* (Baja, light blue, n=34) P0 generation were raised on banana-Opuntia cactus medium. L2 sleep was measured over a 3-hour interval in the F5 generation (after five generations on the cactus diet) to minimize laboratory adaptation epigenetic effects. (E) Total sleep duration. (F) Sleep bout number. (G) Sleep bout length. (H) Waking activity. (E-H) Unpaired two-tailed Student's t-test (**** p<0.0001, *** p<0.001).

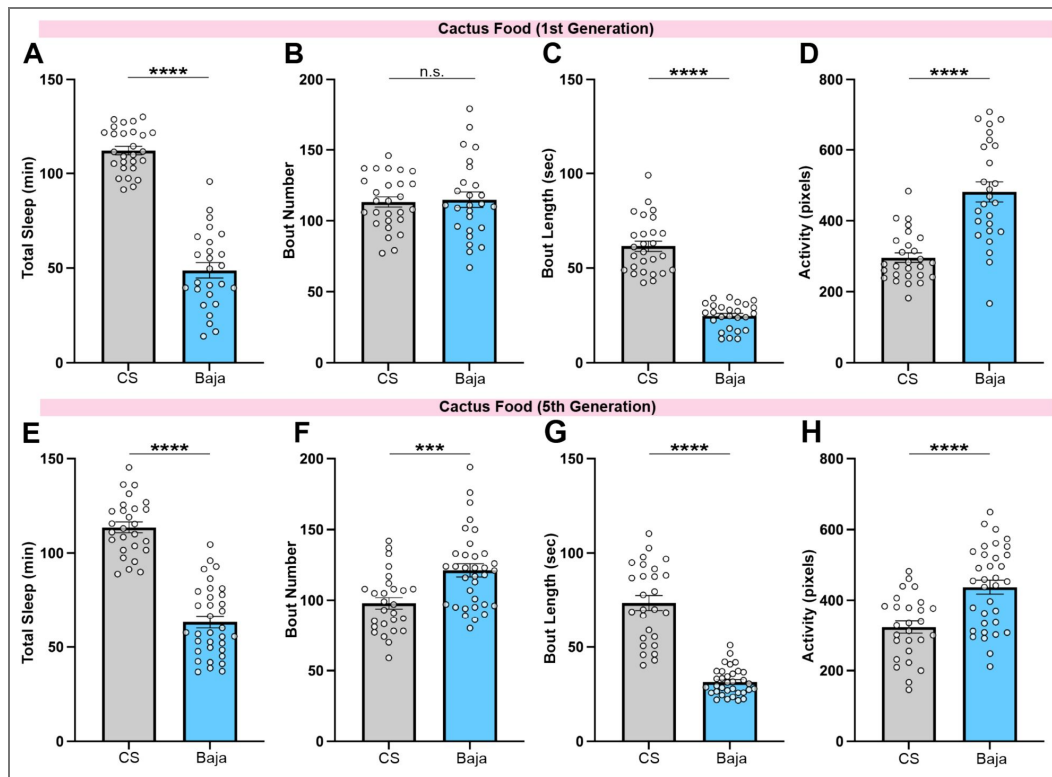
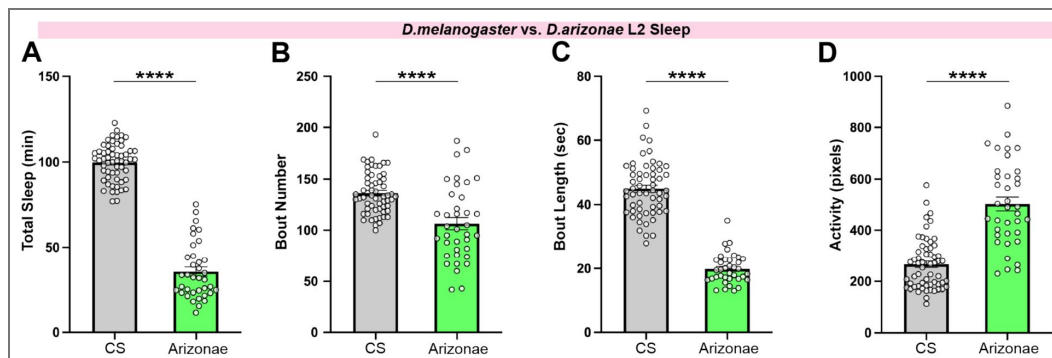


Figure S3. L2 sleep reduction and fragmentation are conserved in a closely related desert species.

(A-D) *D.melanogaster* (CS, grey, n=58) and *D.arizonae* (green, n=37) L2 were monitored for 3 hours to assess sleep. (A) Total sleep duration. (B) Sleep bout number. (C) Sleep bout length. (D) Waking activity. (A-D) Unpaired two-tailed Student's t-test (**** p<0.0001).



Data availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. All raw data will be made available.

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Peer reviews

Joint Public review:

Summary

This interesting work by Shuhao Li and colleagues suggests that developmental sleep and feeding behavior in larval flies is genetically programmed to prepare the animal for adult contingencies, such as in the case of flies living in harsh ecological environments, such as deserts. Thus, the work proposes that desert-dwelling flies such as *Drosophila mojavensis* sleep less and feed more than *D. melanogaster* as larvae, which allows them to feed less and sleep more as adults in the harsh desert conditions where they live. The authors argue that this is evidence for developmental sleep reallocation, which helps the adult flies survive in the desert. In general, their results support this compelling hypothesis, so this work provides a new perspective on how sleep might be differentially programmed across developmental stages according to the requirements of an ecological niche. This work is particularly innovative for several reasons. First, it extends the *Drosophila* sleep field beyond *D. melanogaster* and directly addresses questions about the evolution of sleep that remain largely unexplored. Second, it investigates the possibility that changes in sleep across development may be adaptive, rather than sleep being a static trait. Overall, this work opens new avenues of research, effectively bridges the fields of sleep biology and evolutionary ecology, and should be of broad interest to a general readership. The manuscript is scientifically sound and clearly written for a generalist audience.

There are, however, two important weaknesses that should be addressed. The first is the implicit assumption that all observed behavioral differences are adaptive; this would benefit from a more cautious framing. Second, the manuscript would be strengthened by a more detailed discussion, and potentially additional data, regarding the ecological differences experienced by *D. mojavensis* and *D. melanogaster* at distinct life-cycle stages.

Strengths:

- (1) The study astutely uses desert *Drosophila* species as models to understand how sleep is optimized in a challenging environment. The manuscript is rigorous, experiments are well controlled, the work is very clearly presented, and the results support the main conclusions, which are quite exciting.
- (2) The manuscript examines previously unexplored sleep differences in a non-melanogaster species.
- (3) The study provides evidence that selective pressure can be restricted to specific developmental stages.
- (4) This work offers evolutionary insights into the trade-offs between sleep and feeding across development.

Weaknesses

- (1) The authors should soften interpretations so that it is not assumed that any observed difference between *mojavensis* and *melanogaster* is necessarily adaptive, or evolved due to

food availability or temperature stress.

(2) The study relies on comparisons and correlations. While it seems likely that the observed differences in sleep explain the increased food consumption and energy storage in the larvae of desert flies, demonstrating this through sleep manipulation would strengthen the authors' conclusions.

(3) The question arises regarding whether transiently quiescent larvae are always really sleeping, and whether it is appropriate to treat sleep as a stochastic population-level phenomenon rather than as an individual trait.

(4) The manuscript would benefit from comparative analysis beyond *mojavensis* and *melanogaster*.

(5) A deeper discussion of the ecological differences between the 2 *Drosophila* species would place the results in a broader context.

(6) The feeding parameters used in adults and larvae measure different aspects of feeding, confounding comparisons.

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