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PNAS

# Natural selection against a circadian clock gene mutation in mice

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**Circadian rhythms with an endogenous period close to or equal to the natural light–dark cycle are considered evolutionarily adaptive ("circadian resonance hypothesis"). Despite remarkable insight into the molecular mechanisms driving circadian cycles, this hypothesis has not been tested under natural conditions for any eukaryotic organism. We tested this hypothesis in mice bearing a short-period mutation in the enzyme casein kinase 1ε (*tau* mutation), which accelerates free-running circadian cycles. We compared daily activity (feeding) rhythms, survivorship, and reproduction in six replicate populations in outdoor experimental enclosures, established with wild-type, heterozygous, and homozygous mice in a Mendelian ratio. In the release cohort, survival was reduced in the homozygote mutant mice, revealing strong selection against short-period genotypes. Over the course of 14 mo, the relative frequency of the *tau* allele dropped from initial parity to 20%. Adult survival and recruitment of juveniles into the population contributed approximately equally to the selection for wild-type alleles. The expression of activity during daytime varied throughout the experiment and was significantly increased by the tau mutation. The strong selection against the short-period *tau* allele observed here contrasts with earlier studies showing absence of selection against a Period 2 (*Per2*) mutation, which disrupts internal clock function, but does not change period length. These findings are consistent with, and predicted by the theory that resonance of the circadian system plays an important role in individual fitness.**

circadian rhythms | survival | reproduction | resonance | tau mutation

Circadian clocks are a ubiquitous feature of life on earth, and serve to maintain synchrony of internal physiology with the external 24-h environment. Colin Pittendrigh, one of the founders of chronobiology, hypothesized that natural selection should favor circadian systems to operate in resonance with the external cycle (1, 2). A prediction from this hypothesis is that individuals exhibiting circadian rhythms with frequencies that are not in close resonance with the 24-h cycle should be selected against in nature. The hypothesis was initially supported by laboratory experiments in fly species that lived longer in a 24-h light–dark (LD) cycle than in non-24-h LD cycles (2–4). Stronger support emerged from dyadic competition experiments in batch cultures of cyanobacteria carrying single gene mutations affecting their circadian period (τ). Strains (either wild type or mutant) with a τ similar to the external LD cycle outcompeted strains with a τ different from the *Zeitgeber* (5, 6). Whether periods out of resonance with the external cycle entail a real fitness deficit in a natural setting has not been tested in any of these systems.

The $Ck1\varepsilon^{tau}$ (hereafter defined as the *tau* mutation) is a gain-of-function mutation (7) that accelerates the cellular dynamics of the circadian PERIOD protein (8, 9) and affects circadian behavior and physiology (10). It was first detected in Syrian hamsters (*Mesocricetus auratus*), where it causes τ to shorten with ∼2 h for each copy of the mutant allele (11). In mice, the same mutation shortens the circadian cycle to an almost identical extent (10). As a consequence of the accelerated circadian clockwork, both homozygote *tau* mice and hamsters are unable to entrain to 24-h LD cycles in the laboratory. Because its frequency deviates considerably from the natural 24-h cycle, the *tau* mutation provides an excellent model to study effects of deviant circadian periods on fitness in a natural setting. Here we report the consequences of deviant circadian rhythms in six replicate outdoor populations of mice. These populations were established with the release of mice, all born to two heterozygote parents, in identical enclosures, with ∼49% mutant *tau* alleles in a near Mendelian ratio in each pen. We used s.c. transponders to record each individual's visits to feeders in each enclosure, which allowed us to quantify the rhythm of feeding activity and to keep track of each individual's presence—and, hence, monitor lifespan, mortality, and the *tau* allele frequency in each population.

## Results

**Population Development and Feeding Activity.** Initial mortality directly after release was low because 231 of the 235 mice released were recorded at the feeders at least once. Two weeks after release, only six (2.5%) of the mice were absent. This absence was not biased toward one of the genotypes ($\chi^2 = 0.072$, $P = 0.964$). The six populations gradually increased over time to a total of 868 mice after 14 mo. Mice were live-trapped in March, May, and September 2008 to fit new mice with a transponder and to assess their genotype. The experiment was terminated in January 2009 with the capture of all

### Significance

The circadian clock has evolved to anticipate daily events and is assumed to be important for Darwinian fitness. The endogenous period of the clock runs close to 24 h, permitting accurate entrainment to the natural light/dark cycle. Circadian clocks with abnormal periods are therefore predicted to have negative consequences for fitness. We compared the fitness of mice with deviant circadian periods in populations living in a seminatural environment. Mice with near 24-h "resonant" rhythms survived longer and reproduced more than mice with rhythms shortened by a mutation in the circadian Ck1ε allele. Apart from the fundamental importance of such fitness effects in nature, this finding may have implications for humans subjected to circadian-rhythm deviations under abnormal work and lighting schedules.

Author contributions: K.S., M.W., S.D., and M.H. designed research; K.S., M.W., and M.H. performed research; A.S.I.L. contributed new reagents/analytic tools; K.S. and S.D. analyzed data; and K.S., S.D., A.S.I.L., and M.H. wrote the paper.

The authors declare no conflict of interest.

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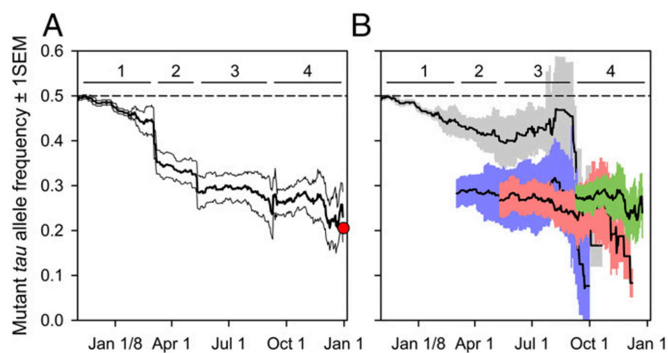


**Fig. 1.** Mutant *tau* allele frequency development in the six replicate populations. (*A*) The mean ± 1 SEM for all cohorts together. The drops in March, May, and September result from the inclusion of new mice after trapping. The dot on the right denotes final allele frequency after trapping all mice at the end of the experiment. (*B*) Mean frequency ±1 SEM for the cohorts separately. Horizontal lines (1–4) indicate the four trapping intervals (see also Tables 1 and 3).

mice. In all trappings, a total of 853 new individuals were caught, genotyped, fitted with transponders, and released, so that analyses are based on a total of 1,088 mice.

**Mutant Allele Frequency and Effects on Adult Survival and Juvenile Recruitment.** The frequency of the mutant *tau* allele gradually decreased in each population from the start of the experiment onward. In the six pens together, the frequency dropped from 49.1% at the beginning to 20.5% at the end of the experiment 14 mo later ($\chi^2 = 898.1$, df = 5, $P < 0.0001$), with steep drops when a new cohort was added after trapping mice (Fig. 1*A*) and subsequent drops in each cohort as it came of age (Fig. 1*B*). The selection against the mutant *tau* allele may thus be attributed to effects on survival of adults or recruitment of new generations by reproduction, or both. We tested for effects on survival by computing for each of the four trapping intervals the number of mice released at the start ($R$) and the number of these still present alive at the end of the interval ($S$). The distributions of the three genotypes among $S$ and $R$ were compared, and absence of a genotype effect on survival was tested by $\chi^2$. Tests were carried out over the first three intervals and their sums (Table 1); in the fourth interval, effects of the genotypes on the distributions at $R$ and $S$ could not be tested because the average survival of all mice ($S/R$) had dropped from 40% to 5%, possibly because of high population densities. Also, the number of homozygote mutants among $R$ had dropped from 20.4% to as far down as 6.6% of the population, such that the expected number among these that survived ($S$) was only 1.8 mice, which is too small for testing by $\chi^2$. Survival fractions over intervals 1–3 were

**Table 1.** **Statistical evidence on *tau* genotype dependence of adult survival and juvenile recruitment in the combined six populations**

| Int. (d) and genotype | *R* | *S* | *S/R* | $\chi^2$ surv | *P* surv | fA* | Y | EY | $\chi^2$ recr | *P* recr |
|---|---|---|---|---|---|---|---|---|---|---|
| Int. 1 (157) | | | | | | | | | | |
| +/+ | 52 | 23 | 0.44 | | | | 74 | 41.4 | | |
| *tau*/+ | 135 | 61 | 0.45 | | | | 64 | 73.7 | | |
| *tau*/*tau* | 48 | 8 | 0.17 | | | | 10 | 32.8 | | |
| All | 235 | 92 | 0.39 | 12.81 | <0.01 | 0.471 | 148 | | 42.78 | <0.0001 |
| Int. 2 (47) | | | | | | | | | | |
| +/+ | 97 | 76 | 0.78 | | | | 102 | 84.6 | | |
| *tau*/+ | 125 | 82 | 0.66 | | | | 70 | 82.3 | | |
| *tau*/*tau* | 18 | 13 | 0.72 | | | | 15 | 20.0 | | |
| All | 240 | 171 | 0.71 | 4.34 | >0.1 | 0.327 | 187 | | 6.67 | 0.035 |
| Int. 3 (114) | | | | | | | | | | |
| +/+ | 178 | 43 | 0.24 | | | | 232 | 229.2 | | |
| *tau*/+ | 152 | 26 | 0.17 | | | | 178 | 177.4 | | |
| *tau*/*tau* | 28 | 3 | 0.11 | | | | 31 | 34.3 | | |
| All | 358 | 72 | 0.20 | 4.21 | >0.1 | 0.279 | 441 | | 0.36 | >0.1 |
| Int. 4 (106) | | | | | | | | | | |
| +/+ | 275 | 16 | 0.058 | | | | 530 | 453.5 | | |
| *tau*/+ | 204 | 9 | 0.044 | | | | 272 | 325.2 | | |
| *tau*/*tau* | 34 | 2 | 0.059 | | | | 35 | 58.3 | | |
| All | 513 | 27 | 0.053 | * | * | 0.264 | 837 | | 30.89 | <0.0001 |
| Ints. 1–3 (318) | | | | | | | | | | |
| +/+ | 327 | 142 | 0.43 | | | | 408 | 328.1 | | |
| *tau*/+ | 412 | 169 | 0.41 | | | | 312 | 353.0 | | |
| *tau*/*tau* | 94 | 24 | 0.26 | | | | 56 | 94.9 | | |
| All | 833 | 335 | 0.40 | 9.94 | <0.01 | | 776 | | 40.16 | <0.0001 |

Data are listed in the table for the three genotypes over four intervals (1–4; Fig. 1) of the study. Headings from left to right are defined as follows: interval (d) and genotype, duration of interval (int.) between trapping in days and genotype; *R*, mice released with tags at the beginning of the interval (including those already tagged before); *S*, tagged mice caught alive at the end of the interval; *S/R*, fraction surviving the interval; $\chi^2$ survival (surv) and *P* surv, test of independence of survival from genotype; fA*, relative mean frequency of the *tau* allele in the population in the interval, calculated from the means of the frequency among R and S; Y, young, untagged mice caught at the end of the interval and assumed born in the interval; EY, expected distribution of genotypes among these young mice on the basis of the fA* and assuming Hardy–Weinberg equilibrium; $\chi^2$ recruitment (recr) and *P* recr, test of the correspondence of the genotype in the prior (EY) and new (Y) cohort. In the bottom four rows, the data for intervals 1–3 are added and analyzed jointly. Note: In interval 4, the strongly reduced initial frequency of homozygote mice (6.6%) combined with the low winter population survival (5.3%) yielded an expected S for this genotype of only 2, too small to allow for $\chi^2$ testing. For these reasons, interval 4 was not included in the overall joint analysis (for intervals 1–3) in the lower part of the table. Genotype dependence of recruitment can however be tested over all four intervals combined and yields $\chi^2 = 108.8$ ($P < 0.0001$).

**Table 2. Cox proportional hazard model on survival of the mice in the release cohort**

| Coefficients | Hazard | Hazard ratio | SE | z | P |
|---|---|---|---|---|---|
| Genotype (*tau*/+) | 0.11 | 1.11 | 0.17 | 0.64 | n.s. |
| Genotype (*tau*/*tau*) | 0.70 | 2.02 | 0.22 | 3.18 | <0.005 |
| Sex (male) | 0.63 | 1.87 | 0.15 | 4.18 | <0.0001 |

Enclosure was added as a random term; $n = 211$, model fit $\chi^2 = 25.9$ (df = 4), $P < 0.0001$; The hazard and hazard ratio values are relative to wild-type and female mice; the positive values for homozygous and male mice indicate a greater risk of death and relative death rate, respectively. n.s., not significant.

significantly affected by genotype (Table 1; $P < 0.01$), with homozygous mutants having the lowest probability of survival. For the cohort initially released, for which the date of birth is known, the effect of genotype on survival had a clear effect on the mean lifespan: +/+ mice, 160.5 d; *tau*/+ mice, 156.2 d; and *tau*/*tau* mice, 105.0 d. Homozygote mutant mice in this cohort exhibited a significantly increased mortality (Cox proportional hazard model; $P < 0.005$). In all three genotypes, male mice lived significantly shorter than female mice ($P < 0.0001$; Table 2); an interaction between genotype and sex was absent ($P = 0.26$).

Genotypic variation in reproduction could not be assessed at the time of birth, but we estimated the variation in recruitment to the population from the genotype distribution of newly appeared young mice that had not yet been fitted with transponders ($Y$) after each interval. The numbers of each genotype were compared with the number expected ($EY$) on the basis of the mean allele frequency in the parent generation in the preceding interval [$(R + S)/2$ for each genotype], assuming that new genotypes were produced in a Hardy–Weinberg equilibrium. This assessment of genotype effects on recruitment includes both differential reproduction and differential mortality in early life. After each interval the number of new wild-type individuals (+/+) exceeded the number expected, whereas the number of new homozygous mutants (*tau*/*tau*) was fewer than expected (Table 1). Together, these data show a highly significant genotype dependence in the rate of production of recruits in intervals 1, 2, and 4 separately and in the combined intervals 1–3 ($P < 0.0001$; Table 1): Both homozygous and heterozygous mutants are underrepresented in the new compared with the old generation. Because both adult survival and recruitment were significantly affected by the *tau* mutant allele, we estimated their total effect on fitness—i.e., the rate of gene propagation, along with the contributions of differential survival and differential recruitment. The effects on fitness were estimated by first computing the instantaneous rate loss in mutant allele frequency by differential survival ($i_s$ d$^{-1}$) and then by the combined effects of differential survival and recruitment together ($i_{sr}$ d$^{-1}$; Table 3). These two values turned out to be –0.0014 and –0.0028 d$^{-1}$, respectively. Hence, effects on survival and recruitment contributed approximately equally to the rate of disappearance of the *tau* allele from the population.

**Effect of the *tau* Allele on Feeding Activity Rhythms.** The transponder records permitted assessment of genotype effects on visits to the feeder and presumed feeding activity. Mice spent an average of 37.4 min (interindividual SD 27.8 min) per day at a feeder. Feeding activity rhythms were variable, both within and between genotypes (Fig. 2 *D*–*F*), but the mean activity patterns were comparable (composite actograms, Fig. 2 *G*–*I*), and there was no significant effect of genotype on average total feeding activity per 24 h (ANOVA, $F = 1.322$, $P = 0.25$; Fig. 3*A*). Under the strong natural LD cycle, periodicity in feeding activity was similar in all genotypes. In the Lomb–Scargle power spectrum (12), averaged over all 14-d intervals within the lifespan of an individual, the period with the highest power was at or close to 24 h for virtually all mice, and the spectra of almost all mice had a second peak at a period <24 h (Fig. 4). We quantified each individual's relative expression of feeding activity during daytime [Diurnality index D (13, 14); *SI Appendix*, *SI Text*]. $D$ varied strongly over time (Fig. 2*J*): In the summer of 2008, $D$ reached levels above zero—i.e., more activity during the day than at night. Throughout the study, homozygote *tau* mutants exhibited significantly more diurnal activity than wild types, with intermediate levels of $D$ in the heterozygotes ($\chi^2 = 94.7$, df = 8, $P < 0.001$; Fig. 3*B*). There was no significant interaction between sex and genotype ($P = 0.92$).

## Discussion

Our data show that the circadian *tau* mutant allele has a profound impact on both components of fitness, adult survival and juvenile recruitment, and affects entrainment of behavioral rhythms' natural LD cycles. The strength of selection against the *tau* mutation is remarkable, and can be estimated by the overall instantaneous

**Table 3. Estimation of the instantaneous rate of disappearance of the *tau* allele and the contribution of differential adult survival and juvenile recruitment**

| Interval $t$ | Days | $R$ | $S$ | $Y$ | $fR$ | $fS$ | $fY$ | $i_s$ | $i_{sr}$ | $i_{pop}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 157 | 235 | 92 | 148 | 0.4915 | 0.4185 | 0.2838 | –0.0010 | –0.0035 | –0.0024 |
| 2 | 47 | 240 | 171 | 187 | 0.3354 | 0.3158 | 0.2674 | –0.0013 | –0.0048 | –0.0031 |
| 3 | 114 | 358 | 72 | 441 | 0.2905 | 0.2222 | 0.2721 | –0.0024 | –0.0006 | –0.0008 |
| 4 | 106 | 513 | 27 | 837 | 0.2651 | 0.2407 | 0.2043 | –0.0009 | –0.0025 | –0.0024 |
| End | | 864 | | | 0.2054 | | | | | |
| Mean | | | | | | | | –0.0014 | –0.0028 | –0.0021 |

Headings from left to right are defined as follows: days, duration of interval between last days of each capture effort when all mice were released and the next capture effort; $R$, total number of mice released (after last interval: killed); $S$, number of individuals among $R$ that were present alive at the end of the interval; $Y$, number of (young) individuals present at the end that were not yet present at the beginning of the interval, i.e., born in the interval; $fR$, relative frequency of the *tau* allele among 2*$R$; $fS$, relative frequency of the *tau* allele among 2*$S$; $fY$, relative frequency of the *tau* allele among 2*$Y$; $i_s$ day$^{-1}$, instantaneous rate of loss in relative *tau* allele frequency by differential survival, $\ln(fS_t/fR_t)/d_t$; $i_{sr}$ day$^{-1}$, instantaneous rate of loss in relative *tau* allele frequency by both differential survival and recruitment, $\ln(fY_t/fR_t)/d_t$. Logic: $i_s$ is evident from the instantaneous rate of mortality. When young are born, the loss of *tau* alleles into the new generation can also be considered an instantaneous rate. Because the parents over time have different frequencies, the new generation carries alleles resulting from both loss due to parent survival and to recruitment. $i_{sr}$ estimates the product of both, which means in terms of instantaneous rates: the sum. The calculation implicitly assumes that the rate of loss due to survival is age-independent. $i_{pop}$ day$^{-1}$, instantaneous rate of overall loss in relative *tau* allele frequency observed in the population, $\ln(fR_{t+1}/fR_t)/d_t$.

rate of population decline in the relative frequency of the mutant allele, which is $i_{pop} = -0.0021\ d^{-1}$ (Table 3), or ∼19.6% per 100 d. This value for strength of selection compares with competition studies of circadian period mutations of Cyanobacteria (*Synechococcus*) maintained in batch culture with wild-type strains, which reported a net disappearance rate of 9.5% per generation (5). Collectively, these disappearance rates suggest that natural selection operates to eliminate genotypes expressing a circadian oscillation that is not in resonance with the period of the earth's rotation.

**Fig. 2.** Effects of *tau* on daily activity in the laboratory and in the field. (*A–C*) Running-wheel activity in the laboratory (double-plot; i.e., *x* axis represents 48 h) of a wild-type (*A*), heterozygous (*B*), and homozygous (*C*) *tau* mutant mouse under LD 12:12 h and constant darkness (DD; day 15 onward; gray shade indicates darkness). *tau/tau* mice do not entrain; *tau/+* mice entrain with a phase advance to the LD cycle. (*D–I*) Activity patterns recorded in outside enclosures for individual wild-type (*D*), heterozygote (*E*), and homozygote (*F*) *tau* mutant mice released on November 2, 2007, until last recorded; and composite actograms for all present wild-type (*G*), heterozygote (*H*), and homozygote (*I*) mice for the entire course of the experiment. Shaded: darkness from civil twilight (ct) at dusk to ct at dawn. (*J*) Diurnality Index *D* over the entire course of the experiment.

**Fig. 3.** Variation in average daily feeding activity and average diurnality index between *tau* genotypes. Feeding activity, expressed in presence (in the number of 1-min bins per day per individual) at the feeders (no effect of genotype; ANOVA, $F = 1.322$, $P = 0.25$) (*A*) and the diurnality index *D* (*B*) estimates from a linear mixed effect model (LME) fitted with sex as fixed effect and date, individual, and enclosure as random effects (effect of genotype with both sexes combined, $\chi^2 = 94.7$, df = 8, $P < 0.0001$).

Our analyses suggested that both adult survival and juvenile recruitment contributed to selection for wild-type alleles. To assess potential differences in recruitment among genotypes, we used the genotype distribution of known mice halfway through a trapping interval to predict the genotype distribution of offspring born in that interval and trapped at the end of it. Recruitment to the next generation encompasses both reproduction itself and juvenile survival until the next trapping effort. Recruitment could have been impaired in homozygous and heterozygous mutants through decreased mating success and lowered fecundity of parents, as well as increased intrauterine or juvenile mortality, both before and after weaning, none of which could be quantified in our field study. We nonetheless think it is useful to distinguish this episode of life history from adult mortality. The pathways through which the mutation affected adult survival likewise still have to be identified. In the initial release cohort—the only cohort for which we could measure lifespan directly—the *tau* mutation reduced lifespan of homozygous, but not of heterozygous, mice. We have no explanation as to why longevity in heterozygous mice was not intermediate; however, the reduced recruitment relative to the expected (*Y*/*EY* in Table 1) shows intermediate values for heterozygote mice over intervals 1–3 (1.24, 0.88, and 0.59 for wild type, heterozygotes, and homozygotes, respectively).

The reduced lifespan in homozygous mice contrasts with laboratory studies of *tau* mutant hamsters kept in continuous darkness, and thus in the absence of an entraining LD cycle, in which the lifespan of male homozygote mutants was increased compared with wild types (15). In another study, heterozygote tau mutant hamsters (expressing a free-running 22-h circadian period in absence of time cues) were kept on a 24-h LD cycle, to which these animals entrained with fractured activity patterns and earlier onset of activity. This aberrant rhythmicity in the 24-h LD cycle was associated with cardiac defects, which did not arise in heterozygotes kept on a 22-h LD cycle (16, 17). The effects were also not observed after ablation of the central circadian pacemaker in the hypothalamus (suprachiasmatic nucleus) or in homozygous hamsters, which do not entrain to modulo-24, and free run across such lighting regimes. Thus, these physiological defects arose in tau hamsters as a consequence of abnormal entrainment and not as a result of a pleiotropic effect of the tau allele, an outcome also consistent with Pittendrigh's circadian resonance hypothesis.

The circadian organization of behavior in the laboratory does not necessarily phenocopy behavior in the field (18). Our behavioral

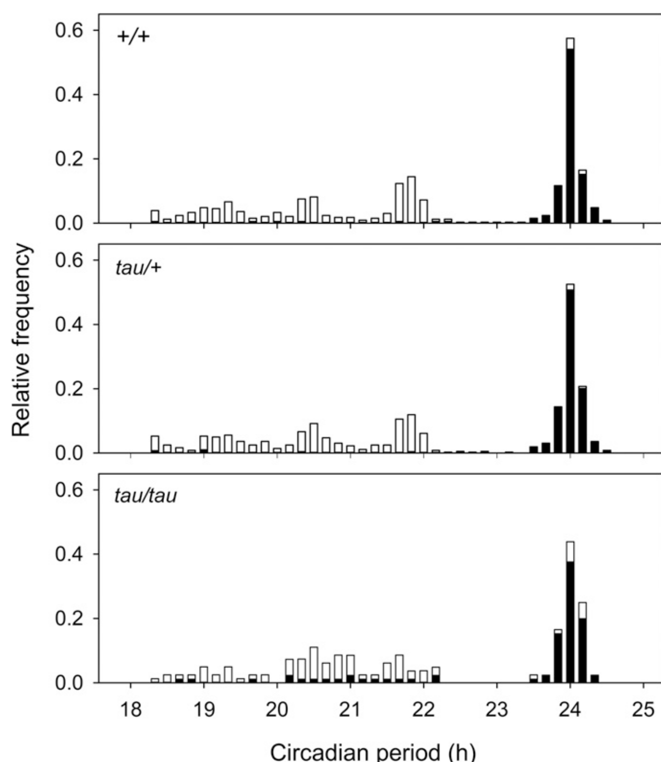


**Fig. 4.** Distribution of the first and second period in feeding behavior. For each individual, these two periods were defined as the highest and second highest peak in the average 14-d Lomb–Scargle (normalized) power spectrum. Each bar shows relative numbers of mice with their first (filled part of each bar) and the second (open part) most prominent rhythm at a specific period length (10-min resolution); $n = 334/332$ (first/second; +/+); $n = 362/360$ (*tau*/+); $n = 85/81$ (*tau*/*tau*).

recordings enabled us to quantify expressed circadian phenotypes, which revealed that most mice had feeding rhythms with a ∼24-h period, but also with a less prominent shorter period. These circadian phenotypes may be attributed to the natural 24-h LD cycle being strong enough to entrain mice of all genotypes, but it is equally possible that natural illumination exerted a strong masking effect on mouse activity. There was, however, a significant contribution of circadian genotype to the extent of diurnality of feeding activity, with homozygous mutant mice being the most and wild-type mice the least diurnal. We observed a clear increase in diurnality index $D$ during the summer months in all genotypes (Fig. 2 *G*–*I*). Increased $D$ may reflect high flexibility in the feeding rhythm, which may facilitate access to the feeders under strong competition. In the release cohort, in which we know the age of all mice, the long-lived mice changed to more diurnal activity patterns at the end of their lives. However, a causal relationship between diurnality and longevity is unlikely here: During the summer, when the long-living mice from the release cohort (the only ones still present from this cohort) became more diurnal, the population density increased. Diurnal behavior under high population densities is consistent with earlier reports of mice maintained under field conditions (14). Activity during the day may have led to a different predation risk between genotypes. However, both nocturnal (great horned owls, *Bubo virginianus*) and diurnal (red-tailed hawks, *Buteo jamaicensis*) predators were video-recorded capturing mice during the experiment, but the extent of the ensuing mortality by these predators could not be quantified. Alternatively, entrainment of animals maintained outdoors may have exacerbated the internal desynchronization between the light-entrained neural pacemaker and that of other body tissues (19, 20), which is known to occur in *tau* mutants. Although breeding in mice may be modulated by photoperiod, we were unable to measure seasonal effects on reproductive success. Seasonal regulation of reproduction may have been attenuated because we used mice with a C57/Bl6 background, which do not express melatonin, a hormone known to be regulated by day length in wild-derived mouse strains and to have antigonadal effects (21).

The strong and persistent effect of the *tau* allele on fitness contrasts with data from a 2-y outdoor enclosure study of survivorship of mice bearing the circadian $Per2^{Brdm1}$ mutant allele. Here, despite laboratory studies showing an array of deleterious physiological consequences for health in $Per2^{Brdm1}$ (22), there was no overall effect on fitness in outdoor conditions. In contrast to the *tau* mutation, the $Per2^{Brdm1}$ mutation does not strongly affect the circadian period and had a negligible effect on activity patterns in outdoor conditions (14). We cannot exclude the possibility that the effects we report for the *tau* mutation may be due to an unknown noncircadian pleiotropic effect, even though the earlier laboratory studies on *tau* mutant hamsters have clearly revealed health consequences as a result of the deleterious impact of inappropriate entrainment to artificial lighting regimes (17).

In conclusion, our data, based on long-term monitoring of replicate seminatural populations of a vertebrate, show strong selection against the *tau* mutation. The pathway of selection is not clear, but it appears to involve both adult survival and juvenile recruitment. It is consistent with the hypothesis proposing a positive role for circadian resonance in fitness, although an unknown pleiotropic effect of the mutation cannot be excluded.

## Materials and Methods

**Setup of Mouse Populations.** Six replicate mouse (*Mus musculus domesticus*, C57/Bl6 background) populations were established in enclosures at the Stony Ford field station in Princeton (W074 40, N40 21). Each population was set up with the release of ∼17 male and 22 female mice per enclosure. In each sex, there was an initial approximate Mendelian ratio of 0.84:2:0.67 (females) and 0.72:2:0.74 (males) of wild-type, heterozygous, and homozygous individuals, respectively, with respect to the *tau* mutation generated in the laboratory of A.S.I.L. (10). All mice were bred from heterozygote parents at the breeding facility at the University of Groningen and raised in constant dim light. At ∼7 wk of age, each mouse received a 90-mg transponder (Trovan ID100, Dorset ID) s.c. The mice were released on November 2, 2007, at 84 ± 0.1 d of age, and littermates were distributed over different enclosures. Each enclosure measured 180 $m^2$ and was fenced by a 1.5-m-high sheet metal wall with three electric fence wires, keeping out terrestrial predators, without eliminating aerial predation. A small (180 × 140 × 70 cm) hay-filled shed was present in each enclosure. The experiment was conducted with permission from the Princeton University Institutional Animal Care and Use Committee (Protocol 1626). All procedures in the experiment were in accordance with national regulations (New Jersey Fish & Wildlife Service).

**Data Collection and Analysis.** To obtain food, mice had to enter one of two feeding stations, thereby passing through a decoder antenna coil (Ø 40 mm; ANTC40, DorsetID). An individual was considered deceased if not recorded for a week (*SI Appendix*, *SI Text*). Mice were fed breeder diet (Mouse Diet 9F; LabDiet) to facilitate population growth, because a very low number of mice may cause unwanted bias in allele frequency change (genetic drift); water was supplied ad libitum. At three stages during the experiment, all mice were trapped at the feeders, and all new mice had a small piece of ear tissue taken for genotyping, were fitted with a transponder, and were weighed and sexed before release. During the first week of 2009, all mice present were trapped and killed by $CO_2$ asphyxiation. All mice were genotyped by using standard PCR techniques (*SI Appendix*, *SI Text*). All records of feeder visits were sorted per individual, converted to binary files with activity in 2-min bins, and further processed with ChronoShop (Version 1.1; written by K.S.). Periodicity was measured by calculating the Lomb–Scargle periodogram (12) and averaging the normalized power spectra over 14-d intervals. The diurnality was defined as $D = (C_D - C_N)/(C_D + C_N)$, where $C_D$ and $C_N$ are the numbers of records per hour from civil dawn to civil dusk, and vice versa (13). Hence, when all activity is expressed at night, $D$ reaches the value of −1, which changes to +1 when activity occurs exclusively in daytime. Differential survival in the release cohort was tested in a Cox proportional hazard model with genotype and sex as fixed effects and enclosure as random effect. Variation in diurnality index $D$ throughout the experiment in all censored mice was tested in a mixed-effect model with date, individual, and enclosure fitted as random effects. All statistics were performed in R (Version 3.1.1; ref. 23).

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