reversal increases sharply as the duration of unstable outer-core field behaviour exceeds this value. The $^{40}Ar/^{39}Ar$ ages, indicating that dynamo instability lasted an order of magnitude longer than this diffusion time, offer the first radioisotopic observation supportive of this claim. It remains to be explored whether, to be successful, a reversal attempt requires an extended, multi-stage dynamo process like that seen not only for the M–B but also in other Cenozoic reversal records[14,26].

Examination of the modern-day non-axial dipole (NAD) field reveals that the global pattern of VGPs that would arise if the axial dipole field were to vanish is not random[27]. In particular, a significant fraction of the Earth's surface encompassing the Pacific, including Tahiti, would experience field directions associated with south VGPs in and around Australia[27]. Because the dynamo is blind to the sign of magnetic flux[1], the three most detailed records obtained from Tahitian lavas are wholly compatible with this simple model. Specifically, north VGPs associated with the R–R Punaruu[15,20] and N–N Big Lost[27] events, as well as the N–R Jaramillo–Matuyama[15,20] reversal, display a distinct preference for the Australasian region early in each record and contain a VGP cluster nearly identical to that of Tahitian M–B lavas shown in Fig. 3. Given the precise ages of the four M–B lava sequences, we can add another Tahitian result to this list. Along with the modern-day NAD-field analysis, these correspondences indicate that a mantle-controlled pattern of flux at the core surface might dominate the field configuration upon initiation of a dynamo instability when almost complete destruction of the axial dipole occurs. That transitional VGPs at this time on La Palma are found mainly elsewhere—over southern South America—lends support to a globally observed, largely non-dipolar early M–B field. Indeed, transitional M–B VGPs obtained from North Atlantic marine sediments[9,28] indicate long-lived residences in this same locality.

Subsequent field behaviour associated with the actual polarity change might be more complex. Apparently, for the M–B transition, a significant fraction of the total 18 kyr of recorded instability elapsed before flux diffusion from the solid inner core allowed the reversal to proceed. □

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Sex increases the efficacy of natural selection in experimental yeast populations

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Why sex evolved and persists is a problem for evolutionary biology, because sex disrupts favourable gene combinations and requires an expenditure of time and energy[1]. Further, in organisms with unequal-sized gametes, the female transmits her genes at only half the rate of an asexual equivalent (the twofold cost of sex)[2]. Many modern theories that provide an explanation for the advantage of sex incorporate an idea originally proposed by Weismann more than 100 years ago: sex allows natural selection to proceed more effectively because it increases genetic variation[3–5]. Here we test this hypothesis, which still lacks robust empirical support, with the use of experiments on yeast populations. Capitalizing on recent advances in the molecular biology of recombination in yeast, we produced by genetic manipulation

strains that differed only in their capacity for sexual reproduction. We show that, as predicted by the theory, sex increases the rate of adaptation to a new harsh environment but has no measurable effect on fitness in a new benign environment where there is little selection.

The Weismann effect is expected whenever fitness correlations between loci are negative. Under these circumstances, the net effect of sex is to bring together favourable mutations, separate favourable mutations from harmful mutations, and concentrate harmful mutations, all of which can increase the efficacy of selection. Negative fitness correlations can arise from epistatic interactions between loci and through the effects of finite population size[4]. In diploid organisms, sex can also increase the variance of fitness by the segregation of alleles at individual loci or because of selection on the transient haploid phase[6,7].

If the Weismann effect is important, then adaptation to new evolutionary challenges should proceed faster in sexual populations than in asexual populations. Experiments with yeast and *Chlamydomonas*[8–11], along with work on other taxa[12,13], have largely supported the hypothesis, although studies so far are subject to several possible criticisms. Sexual reproduction in microbial model systems is normally manipulated by starvation, which means that asexual and sexual replicates experience different selection regimes. Starvation in both *Chlamydomonas* and yeast is known to increase mutation rates[14,15]. One study with *Chlamydomonas* avoided such treatment differences, but at the expense of the association of sex with large-scale genetic introgression[10]. These problems will tend to increase genetic variation, so these manipulations might have significant effects on the course of evolution that are independent of sexual reproduction. Advances in our understanding of the molecular mechanisms of meiosis in yeast allow us to circumvent these problems by genetically engineering an asexual strain that can be treated identically to the sexual wild type. This allows us to study the net effect of sex itself on the rate of adaptation.

Yeast cells grow vegetatively when supplied with sufficient nutrients; however, if starved, diploids undergo sporulation (meiosis) and produce four haploid spores encapsulated within an ascus[16]. Diploid yeast are heterozygous (**a**/α) at the mating-type locus (*MAT*); the ascus therefore contains two spores of each mating type. When conditions improve, these spores germinate and mate with a spore of the opposite mating type. Yeast are isogamous (each spore contributes equally to the resulting diploid zygote) and so this system has no twofold cost of sex. Apomictic strains of yeast that produce pairs of diploid spores (dyads) instead of four haploid spores (tetrads) have occasionally been recovered from natural populations[17].

We constructed an apomictic (asexual) strain of yeast that differs from the wild type in its capacity for recombination, random assortment and syngamy by deleting two genes required for normal recombination and meiosis. *SPO11* encodes an endonuclease that initiates crossover events by making double-stranded breaks in chromosomes: in its absence meiotic recombination does not occur[18]. *SPO13* determines whether a cell goes through one or two meiotic divisions by altering the sister-chromatid cohesion process[19]; in its absence only the second non-reductive meiotic division is achieved, resulting in the production of two diploid spores[20]. Because chiasmata are required for the stabilization of chromosome segregation, non-functional mutations of *SPO11* would normally lead to aberrant chromosomal segregation, but this phenotype is rescued if *SPO13* is also non-functional; this leaves the asexual double mutant fully fertile, producing diploid spores that are genetically identical to the parent cell (refs 18, 21 and see below). We deleted both of these genes by using standard yeast engineering techniques, and replaced *SPO13* with the bacterial *kanMX4* G418 resistance gene to provide a marker to distinguish the asexual strain[22]. The engineered asexual strain is thus not only able to sporulate but also genetically identical to the sexual strain except at these two loci.

Weismann's hypothesis predicts that sex will lead to an increase in mean fitness when there is selection, but not otherwise. We tested this hypothesis by comparing the rate of adaptation of replicate sexual and asexual yeast populations (eight each) when exposed to one of two environments: a new benign chemostat environment in which growth was limited by glucose concentration (0.08%), or a harsher chemostat environment with the same glucose concentration but in which the temperature was raised from 30 to 37 °C, and where osmolarity was elevated from 0 to 0.2 M NaCl. The carrying capacities were about 3×10^7 and about 3×10^6 cells ml^{-1} under the benign and harsh conditions respectively, which reflected the relative stresses experienced by these yeast in the two environments.

SPO13 and *SPO11* are expressed only during meiosis[18,20], and the *kanMX4* marker has previously been shown to have a negligible fitness effect in yeast when propagated in glucose-limited chemostats[23]. However, to rule out any unexpected fitness effects of engineering we allowed the ancestral sexual and asexual strains to compete under both the benign and harsh environments. The average intrinsic exponential growth rate (fitness) advantage of the sexual strain over the asexual strain in both environments was similar and very near zero (benign, 0.003 ± 0.0016 (mean ± s.e.m.); harsh, 0.003 ± 0.0023; $n = 8$). We also checked that the manipulation had not affected sporulation efficiency and found that the proportion of cells that produced spores was very similar in both the ancestral sexual and engineered asexual strains (sexual, 76 ± 4% (mean ± s.e.m.); asexual, 73 ± 4%; *t*-test, $P = 0.62$).

Replicate experimental populations of the sexual and asexual strains were each initiated from a single colony and subjected to regular cycles of vegetative growth punctuated by sporulation. Each vegetative growth phase lasted 4 days and took place in about 33 ml of medium in a shaking chemostat. Chemostat generation times (determined by nutrient in-flow rate) were set at about 2.2 h and about 4.0 h for the ancestral strains in the benign and harsh environments, respectively. This meant that in the benign environment there were about 50 mitotic generations between each sporulation episode, and in the harsh environment about 25. To induce sporulation the medium was replaced with a 1% potassium acetate solution; all populations were then incubated without the addition or removal of medium for 7 days at 30 °C (ref. 24), and subsequently removed from the chemostat vessels. Mating after germination normally occurs within asci, and yeast would therefore naturally inbreed; however, to promote outbreeding and to destroy unsporulated cells, the yeast cultures had their asci and cell walls softened with the enzymes β-glucoronidase and lyticase. They were then placed in a 1% solution of the surfactant sodium dodecyl sulphate to lyse unsporulated cells, followed by sonication to release and thoroughly mix the spores. Germination was triggered when the yeast were returned to their normal growth medium, after which mating occurred in the sexual strains. We estimated the level of outbreeding obtained under these conditions by crossing the sexual strain, a uracil auxotroph, with a strain that was genetically identical except that it was a lysine auxotroph. We found that 86 ± 3% (mean ± s.e.m., $n = 8$) of the progeny were outcrossed. Both experiments were run for about 300 vegetative generations, equivalent to five (benign environment) or ten (harsh environment) cycles of growth and sporulation.

We estimated the changes in fitness of the populations over the course of the experiment by allowing samples from the replicate populations to compete against an ancestral strain (yeast can be stored indefinitely at −80 °C in glycerol) under conditions identical to those in the experiment. In these competition assays we determined the proportion of each strain using selective plating at the start and then again after an average of 30 mitotic generations. A

measure of relative fitness under continuous growth was obtained by subtracting the initial from the final natural logarithms of the ratios, and the relative fitness per mitotic generation was found by dividing this figure by the number of generations in each particular competition assay. Confidence limits were calculated taking into account the binomial sampling variance.

We were unable to detect any change in the fitness of either the sexual population or the asexual population in the benign environment (Fig. 1). The overall average cell density neither differed significantly between strains nor changed significantly over the course of the experiment.

In contrast with the experiment in the benign environment, the relative fitness of yeast populations exposed to the harsh environment increased markedly over the course of the experiment. We define relative fitness here as the natural logarithm of population growth rate relative to the ancestral strain (which therefore has a relative fitness of zero). In the asexual lines, relative fitness increased from 0 to 0.59, whereas for the sexual lines the increase was from 0 to 0.68. These figures are equivalent to increases in population growth rate of 80% and 94%, respectively. Inspection of the data and exploratory analysis indicated that fitness was beginning to reach a plateau towards the end of experiment and was therefore analysed with a nonlinear mixed-effect model[25],

$$w_i(t) = a_{1i}[1 - \exp(-A_{2i}t)] + \varepsilon_i(t)$$

where $w_i(t)$ is the relative fitness of replicate i at time t. The nonlinear function, which is constrained to be zero at time $t = 0$, is specified by replicate-specific asymptote (a_{1i}) and rate ($a_{2i} = \ln(A_{2i})$) parameters, each of which in the full model is described by the sum of statistical random and fixed components. The random component reflects differences between replicates, and the fixed component reflects the treatment effects of sexual versus asexual lines. $\varepsilon_i(t)$ is the within-group variance assumed to be independently and normally distributed with zero mean. After simplification of the model, the random effect was confined to the asymptote parameter a_1 and the fixed effect was restricted to the rate parameter a_2 (although an alternative model with the treatment effect on a_1 was nearly as informative). We tested for the significance of sexual versus asexual reproduction by removing the treatment effect, which was highly significant (log-likelihood ratio 12.6, $P = 0.0004$). The data and fitted model are shown in Fig. 1.

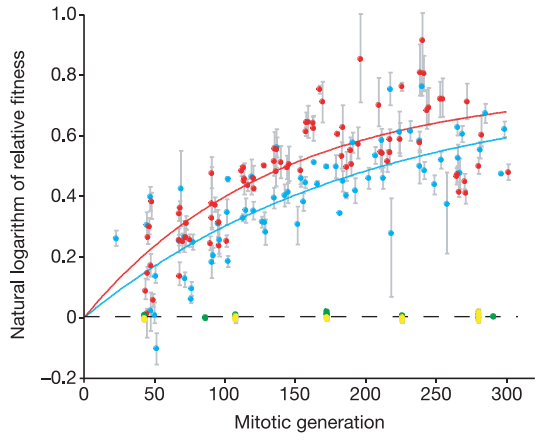

Figure 1 The change in natural logarithm of fitness of asexual and sexual populations of yeast in benign and harsh environments. Points show fitness measurements for individual populations with twice log-likelihood error bars (these approximate 95% confidence limits); the error bars for the benign treatment are plotted but are mostly too small to be discriminated. The fitted model for the harsh environment is plotted for asexual (blue) and sexual (red) treatments (parameters: $a_1 = 0.761$, a_2(asexual) $= -5.287$, a_2(sexual) $= -4.901$). Yellow symbols, asexual strains in the benign environment; green, sexual in the benign environment; blue, asexual in the harsh environment; red, sexual in the harsh environment.

In the new benign environment there was no detectable adaptation over the course of the experiment, and thus no detectable selection for beneficial mutations. There would still have been purifying selection clearing deleterious mutations as they arose. In other experiments it has been estimated that deleterious mutations decrease mean fitness in yeast by about 0.002% in each generation[26]. At mutation–selection balance, selection need only increase fitness by this amount to prevent the accumulation of maladaptive variants. This is a very small value, and any difference there might have been in the efficacy with which sexual and asexual populations cleared their mutations would have been undetectable in our experiment.

By contrast, in the harsh environment, relative fitness increased markedly for both the asexual and sexual populations; after 100 generations the intrinsic rate of increase in the asexual populations exceeded the ancestor by 0.3, but the equivalent figure for the sexual populations was 0.4. Applying Fisher's Fundamental Theorem of Natural Selection this indicates that in the first 100 generations of the experiment there must have been genetic variance in fitness, upon which selection could act, of about 0.003 and 0.004 per generation in the asexual and sexual populations, respectively. This is consistent with the expectation from Weismann's hypothesis that the maintenance of sex is associated with increased variance in fitness. The difference in fitness between the sexual and asexual populations after 100 generations, and indeed throughout most of the experiment, is about 0.1. Although this may not seem a very great advantage, the geometric growth process underlying it quickly leads to large differences in cell numbers. Thus in the 25 mitotic generations between episodes of sex in our experiments, a sexual individual could expect to leave about 12-fold ($e^{0.1\times25}$) as many descendents as an asexual individual. We can also ask whether the rates of evolution in our experiments are unrealistically high. This does not seem to be so, because the 0.3–0.4% variation in fitness we observed is in the lower range of estimates for natural populations of various plants and animals (0.1–30%, with typical values likely to be between 1% and 10%)[27].

Recombination increases the efficacy of natural selection when fitness correlations between loci are negative[4,5]. There are at least two non-exclusive reasons why negative correlations might have occurred in our experiments: epistatic interactions between new mutations, and the effects of finite population size. Finite population size is significant in circumstances in which sex allows adaptive mutations that have occurred in different lineages to be brought together and to increase in frequency simultaneously, rather than their being forced to spread sequentially as they would in an asexual population[28]. Further genetic work to isolate and characterize the mutations responsible for the adaptation to the harsh environment is needed to distinguish between these possibilities.

Meiotic recombination has been seen by many theoreticians as the most important aspect of sex[2], but other effects of sex might also have contributed to the increased rate of adaptation. In diploids the segregation of alleles at a locus can be associated with increased variance in fitness, particularly in populations with some inbreeding (as occurred in our experiments)[7]. In addition, the asexual strains would not have had a brief haploid phase upon which selection could act[6]. In some models of selection with fluctuating epistasis it is possible for sex to increase mean fitness directly, without an effect on the variance[29]. We think that this is unlikely to explain our results: although the populations were exposed to two separate environments (one for vegetative growth and one for sporulation), sex occurred every cycle rather than between each

transition as the model requires. Finally, it is conceivable that the engineered knockouts in the asexuals had some pleiotropic effect of reducing mutation rates.

Our results indicate that sexual reproduction can provide a selective advantage during adaptation to new environments, and these data are consistent with Weismann's ideas. They are an advance on earlier studies because by manipulating sexual status genetically we have mimicked the natural situation more closely and have excluded a variety of possible confounding factors. In extrapolating from experiments with yeast and other single-celled organisms to multicellular organisms, at least two points need to be considered. First, yeast gametes are of equal size (isogamous) and hence the classical twofold cost of sex does not apply to these organisms. There are still costs to sex (see above), but they will be less than in anisogamous species. Second, empirical studies of deleterious mutations in yeast indicate that their effects on fitness are significantly lower than in 'higher' eukaryotes. Therefore one of the major postulated advantages of sex, the removal of epistatically interacting deleterious mutations, will be less important. Population experiments with fast-replicating microorganisms have been very valuable in testing different ideas about the maintenance of sex, and a challenge now is to understand the nature of the mutations that underlie adaptation and to extend these techniques to larger plants and animals. □

Methods

Additional details are given in Supplementary Methods.

Strain construction

We subcultured a Y55-derived haploid α, *ho, ura3Δ* colony and switched mating types[24] to yield isogenic *MAT***a** and α lines. We employed polymerase-chain-reaction-based gene targeting to replace *SPO13* with the *kanMX4* module[22] in the α strain and to delete the *SPO11* locus from the **a** strain (by using two-step uracil plus/minus selection). Haploids were mated to produce a strain heterozygous at only the *SPO11*, *SPO13* and *MAT* loci; this strain sporulated normally and produced four viable spores. MGA1 {**a**, *ho, ura3Δ, spo11Δ, spo13Δ::kanMX4*} and MGS1 {α, *ho, ura3Δ*} were isolated from one tetrad, and MGA2 {α, *ho, ura3Δ, spo11Δ, spo13Δ::kanMX4*} and MGS2 {**a**, *ho, ura3Δ*} from another. Mating yielded strains with identical histories of genetic engineering and isogenic at all but the *SPO11* and *SPO13* loci. Lysine auxotrophs were selected on 5% α-aminoadipic acid[24].

Media and growth

The basic mitotic growth medium consisted of 0.17% yeast nitrogen base without C, N or amino acids (Sigma), 0.5% ammonium sulphate, 0.08% glucose, 20 mg l^{-1} uracil (Sigma). Continuous culture occurred in a replicated chemostat unit (Infors-HT); flasks were shaken at 125 r.p.m. with independent aeration. The dilution rate averaged 0.31 h^{-1} for the benign treatment and 0.17 h^{-1} for the harsh treatment. Sporulation medium comprised 1% potassium acetate and 5 mg l^{-1} uracil. 'Outcrossing solution' (10 ml; 40 U ml^{-1} lyticase (Sigma), 1,107 U ml^{-1} β-glucuronidase (Sigma), 50 mM dithiothreitol (Sigma); all filtered at 0.2 μm) was added to the cultures and incubated overnight at 37 °C, with shaking. Cross-contamination was checked by using the G418 resistance marker and by visual inspection of the spore structures; it was found only in one case when a sexual population invaded an asexual population in the harsh treatment. Samples (25 ml) were taken from the outflow just before sporulation; they were condensed and stored at −80 °C in 15% v/v glycerol for subsequent fitness estimation.

Outcrossing assay

Eight populations were initiated with approximately equal frequencies of the diploid lys^- and ura^- strains. Control populations with single or haploid strains were also initiated, and all were grown in chemostats for about ten generations, sporulated, and subjected to the outcrossing treatment described above. The amino-acid requirements of the progeny allowed the proportion of each genotype to be estimated: the mean inbreeding coefficient (Wright's *F*) of replicate populations (more than 1,500 sampled colonies) was 0.13 ± 0.03 (mean ± s.e.m.). No haploid (unsporulated) cells survived the treatment.

Fitness assay

Difference in resistance to G418 toxin allowed us to distinguish between competitors. Confidence limits were placed around each fitness estimate with a binomially based maximum-likelihood model. Replicates of individual competition assays converged on very similar estimates (within support limits of one another). Initial competitions were against the ancestor; by generation 150 the fitness of the derived populations under the harsh treatment had increased to the point at which ancestral strains were driven below detectable frequencies in competition assays. We therefore marked a derived sexual strain with G418 resistance and, after estimating the fitness effects of marking (−0.082 ± 0.0090, mean ± s.e.m., $n = 8$), used this as a competitive standard. The competitions were conducted over an average of 12 (harsh) and 50 (benign) generations. In every case, final fitness estimates were adjusted to take into account the relative fitness of the standard competitor; in this way all fitness estimates were rendered directly comparable.

Statistical analyses

Data were analysed with the R statistical package (http://www.r-project.org/) on the basis of the model shown in the main text. If w_{ij} is the jth measurement (at time t_{ij}) of the fitness of line i, then the statistical model can be written

$$w_{ij} = a_{1i}(1 - \exp(-\exp(a_{2i})t_{ij})) + \varepsilon_{ij},$$

$$\begin{bmatrix} a_{1i} \\ a_{2i} \end{bmatrix} = \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} + \begin{bmatrix} b_{1i} \\ b_{2i} \end{bmatrix} = \boldsymbol{\beta}_k + \mathbf{b}_i,$$

$$\mathbf{b}_i N(\mathbf{0}, \boldsymbol{\Psi}), \quad \varepsilon_{ij} N(0, \sigma^2).$$

The fixed effects, β_k, represent the average value of a sexual ($k = 1$) or asexual ($k = 2$) population. The random effects, b_i, are normally distributed with a mean of 0 and a variance–covariance matrix ψ, and are assumed to be constant in time and independent for different lines. The within-group variance, ε_{ij}, is normally distributed with mean zero and variance σ^2, and is independent for different i, j and independent of the random effects. Following ref. 25 we fitted the full random effects model and examined the fitted variance–covariance matrix to see whether all terms were necessary. The random effect b_{2i} was small and correlated with b_{1i}; its removal made little difference to the fit of the model. We therefore restricted the random effect to a_1. The data could be explained almost equally well by assuming that the treatment influenced a_1 or a_2, with an effect on the latter providing a slightly better fit. Allowing both parameters to depend on treatment did not significantly improve the fit (or produce a better Akaike's Information Criteria (AIC) score) compared with either single-parameter model.

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Functional consequences of a *CKIδ* mutation causing familial advanced sleep phase syndrome

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Familial advanced sleep phase syndrome (FASPS) is a human behavioural phenotype characterized by early sleep times and early-morning awakening[1]. It was the first human, mendelian circadian rhythm variant to be well-characterized, and was shown to result from a mutation in a phosphorylation site within the casein kinase I (CKI)-binding domain of the human *PER2* gene. To gain a deeper understanding of the mechanisms of circadian rhythm regulation in humans, we set out to identify mutations in human subjects leading to FASPS. We report here the identification of a missense mutation (T44A) in the human *CKIδ* gene, which results in FASPS. This mutant kinase has decreased enzymatic activity *in vitro*. Transgenic *Drosophila* carrying the human *CKIδ-T44A* gene showed a phenotype with lengthened circadian period. In contrast, transgenic mice carrying the same mutation have a shorter circadian period, a phenotype mimicking human FASPS. These results show that CKIδ is a central component in the mammalian clock, and suggest that mammalian and fly clocks might have different regulatory mechanisms despite the highly conserved nature of their individual components.

Phosphorylation has a central role in the regulation of circadian clocks[2–5]. Multiple points of regulation have been proposed, including the nuclear import and export of circadian proteins, and the active/inactive states of these proteins, but the details of this system are not well understood[6–8]. In *Drosophila*, Per and Tim are two core proteins of the circadian clock. Doubletime (Dbt) and casein kinase II (CKII) phosphorylate Per, and Shaggy (Sgg) phosphorylates Tim[9–13]. The Syrian hamster *tau* mutation was identified in the gene for casein kinase Iε (*CKIε*), a mammalian homologue of *Drosophila dbt*[14]. The known mutations in *dbt*, *CKII*, and *CKIε* lead to hypophosphorylation *in vitro*[11,12,14,15]. However, the different mutations can give rise to different phenotypes *in vivo*, with longer or shorter circadian periods (τ).

FASPS is a behavioural phenotype manifest by early sleep times, early-morning awakening, and a short τ (ref. 1). It was shown to result from mutation in a phosphorylation site within the CKI-binding domain of the human (h) PER2 protein. To gain a deeper understanding of the mechanisms of human circadian regulation, we set out to identify mutations in human subjects that lead to FASPS. We report here the identification of a mutation in the human *CKIδ* gene (also known as *CSNK1δ*), which causes FASPS in humans. This mutation results in reduced kinase activity *in vitro* and leads to a shorter circadian period in mice, but a longer period in *Drosophila*.

The proband of this study was noted by one of the authors (R.E.S.) to have FASPS. Further evaluation of this subject's family revealed autosomal-dominant transmission of the behavioural trait. Fifteen family members, spanning three generations, were separately interviewed for their typical work and vacation sleep–wake schedules by two of the authors (C.R.J. and R.E.S.). Five individuals definitely affected with FASPS (age range 20–65 yr, mean 41 yr) were identified (Fig. 1a). In the absence of competing psycho-social demands, both the average sleep onset time (18:12 ± 1.4 h versus 23:24 ± 1.1 h) and final wake time (04:06 ± 0.7 h versus 08:00 ± 1.6 h) of these subjects were significantly earlier ($P < 0.0001$) than the nine unaffected family members. Affected individuals reported the onset of FASPS somewhere between early childhood to the mid-teen years.

Clinical features or a history of depression were found in three of the FASPS subjects, two of who had mildly elevated Beck Depression Inventory scores of 12 (ref. 16). A tendency towards winter depression was reported by a fourth FASPS subject. However, early-morning awakening due to depression was considered an unlikely explanation for FASPS in this family because the affected individuals showed little difficulty initiating or maintaining sleep, and had good energy levels in the morning (subjectively assessed). In addition, the young age of FASPS onset in these individuals was well below the age at which the typical early-morning awakening develops in depression[17].

While screening candidate genes for circadian mutations (Supplementary Information), an A-to-G change was identified in the DNA sequence of *CKIδ*, which causes a threonine-to-alanine alteration at amino acid 44 in the protein (Fig. 1b). This

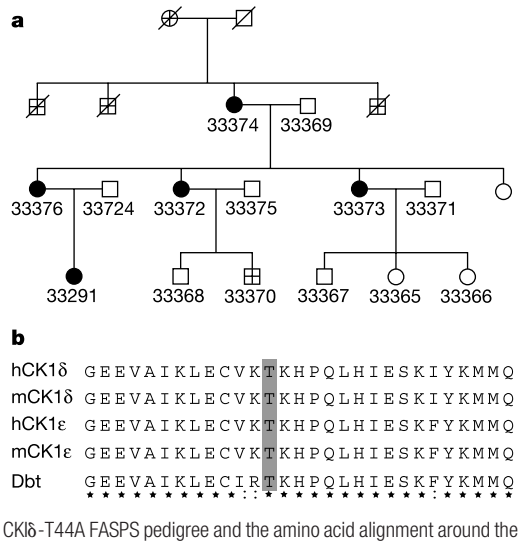

Figure 1 CKIδ-T44A FASPS pedigree and the amino acid alignment around the mutation. **a**, FASPS kindred 5231. Circles represent women, squares denote men, filled circles and squares show affected individuals; empty circles and squares show unaffected individuals. The individual marked with a cross is 'probably affected' but was conservatively classified as unknown. Diagonal lines across symbols indicate deceased individuals. **b**, Alignments for *Drosophila* Dbt and mouse (m) and human (h) CKIδ and CKIε proteins. The T44A mutation is highlighted.