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GENETIC ANALYSIS OF MORNINGNESS AND EVENINGNESS

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ABSTRACT

We studied the influence of genetic factors on individual differences in morningness-eveningness in a sample of Dutch twin families. Data were collected from adolescent twins (mean age 17.8 yr) and their parents (mean age of fathers 48.0 yr and of mothers 46.0 yr) and a sample of older twins (mean age 46.5 yr). Scores on morningness-eveningness were rated on a 5-point scale. Parents were more morning oriented than their children, and women were more morning oriented than men. With a twin-family study, separation of genetic and environmental influences on variation in morningness-eveningness is possible. Including parents and older twins in the study makes it possible to explore generation differences in these effects. The correlation between monozygotic twins was more than twice the correlation between dizygotic twins. This indicates that genetic effects may not operate in an additive manner. Therefore, a model that included genetic dominance was explored. Biometrical model fitting showed no sex differences for the magnitude of genetic and environmental factors. The total heritability—the sum of additive and nonadditive genetic influences—for morningness-eveningness was 44% for the younger generation and 47% for the older generation. How-

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INTRODUCTION

Circadian rhythms are biological phenomena that oscillate with an endogenous period of approximately 24h. These rhythms include body temperature, various hormone levels, the number of immune cells in blood, and the sleep-wake cycle, among others. Circadian regulation is an almost universal function in physiology. Essentially all organisms use an endogenous timekeeping system to control 24h rhythms in physiology and behavior. Studies in organisms as diverse as plants, insects, and mammals have demonstrated the importance of genetic factors in generating the circadian rhythm (1,2).

It has been suggested that circadian rhythmicity in humans is controlled by a self-sustaining oscillator mechanism, as well as by exogenous factors such as the light-dark cycle, level of physical activity, ambient temperature changes, and occasionally other time cues, including the knowledge of time (3,4). Circadian rhythmicity has been demonstrated in several variables, among them body temperature, cortisol, and melatonin. Interindividual variation in the phase of these and other circadian rhythms has been attributed to factors such as age, gender, and especially morningness-eveningness.

Morningness-eveningness represents the interindividual variation in endogenous phase and is reflected in the individual preference for sleep-wake timing. Briefly, a so-called morning-type person (a "lark") is someone who gets up easily and is more alert in the morning than in the evening, has a hard time sleeping late, and falls asleep quickly in the evening. Evening-type persons ("owls"), on the other hand, are more alert at night, are able to sleep late in the morning, and take a long time to fall asleep at night (5). Most individuals, however, occupy a scale position somewhere between the morning-type and evening-type extremes and can be described as neither type (6).

The endogenous nature of the morning-type versus evening-type difference has been corroborated by measurements of various physiological variables under strictly controlled, so-called constant routine conditions (7–9). The phase of the core body temperature, subjective alertness, and melatonin rhythms occurred significantly earlier in morning-type than in evening-type individuals. Many other studies conducted under unrestricted routines also revealed differences in the phasing of temperature and cortisol circadian rhythms (10) Linkowski and co-workers (11,12) measured the 24h cortisol profile in 11 monozygotic and 10 dizygotic twin pairs. A genetic effect was demonstrated for the timing of the

nadir. In contrast, the timing of the acrophase (peak time) of the cortisol rhythm appeared to be controlled primarily by environmental influences.

To investigate morningness-eveningness, we carried out a twin-family study. In 1993, data on morningness-eveningness were collected from a mailed survey of monozygotic and dizygotic adolescent twins and their parents. In a twin design, the separation of genetic and environmental variance is possible because monozygotic (MZ) twins share 100% of their genes and dizygotic (DZ) twins share, on average, 50% of their genetic makeup. If a trait is influenced by genetic factors, MZ twins should resemble each other to a greater extent than DZ twins. When DZ correlation is greater than twice the MZ correlation, this suggests a portion of the resemblance between twins is influenced by the environment they share. Parents and offspring also share 50% of their additive genetic variance, as well as a common family environment. Including parents of twins in the design makes it possible to examine the presence of assortative mating, in this case, nonrandom choice of mating for morningness-eveningness. Furthermore, by including parents in the study, generation differences in genetic architecture can be explored. These generation differences may consist of differences in heritability or of differences in the genes that are expressed as subjects grow older. To distinguish between these two alternatives, data from a second group of twins, the same age as the parents, were added to the design (13).

SUBJECTS AND METHODS

This study is part of a longitudinal questionnaire study that began in 1991 that assesses families with adolescent and young adult twins every 2 years. The data presented in this article came from the 1993 survey, which was sent both to families who had also participated in the study in 1991 (49% of the sample) and to newly recruited families. Addresses of twins were obtained from City Council registries (14).

In 1993, subjects received an 18-page booklet containing a large number of personality inventories, items about zygosity, schooling, socioeconomic status, family structure, health, and lifestyle. One specific question was, "Are you a morning-active person or an evening-active person?" The question had five answer categories: (1) morning active, (2) moderately morning active, (3) neither, (4) moderately evening active, and (5) evening active.

For this study, two data sets were used. The total number of participating families in the first data set was 1974. After excluding 100 families in which the parents of the twins were not the biological parents, 1874 families remained. There were 1566 fathers (mean age 48.0 yr, SD 5.4 yr), 1726 mothers (46.0 yr, SD 5.1 yr), and 3477 twins (17.8 yr, SD 3.1 yr) who completed the questionnaires. For the majority of the twin pairs, zygosity was determined from questions about physical similarity and confusion in identifying the twins by family members, friends, and strangers. For 216 same-sex twin pairs, information on

their zygosity was available from the blood group and/or DNA polymorphisms. The agreement between zygosity diagnosis from questionnaire and DNA data was 93%. Data on morningness-eveningness were available for 277 monozygotic male twin pairs (MZM), 228 dizygotic male twin pairs (DZM), 400 monozygotic female twin pairs (MZF), 296 dizygotic female twin pairs (DZF), and 449 opposite-sex twin pairs (DOS). There were 1650 complete twin pairs (see Table 1), 177 twin pairs in which only one twin answered the question, and 40 families in which only one or both of the parents participated. For 7 families, no data were available on morningness-eveningness.

The second sample consisted of a group of adult twins with parents of the same age as the parents of the first sample (15). Data on morningness-eveningness were available for 26 monozygotic male twin pairs (MZM), 14 dizygotic male twin pairs (DZM), 35 monozygotic female twin pairs (MZF), 28 dizygotic female twin pairs (DZF), 21 opposite-sex twin pairs (DOS), and 68 twin pairs in which only one twin answered the question. Mean age for those twins was 46.5 yr (SD 6.6 yr). Zygosity was determined on the basis of DNA typing of all twins of the second data set.

STATISTICAL ANALYSES

Scores on morningness-eveningness were rated on a 5-point scale. To test for sex and generation differences, Mann-Whitney tests were performed. To investigate the inheritance of morningness-eveningness, the character was considered to have an underlying continuity with thresholds. The underlying continuous variable has been called the *liability.* The continuous variation of the liability is both genetic and environmental in origin. It may be a compound of several different psychological or developmental processes, but it is not necessary to know how these are combined to give the liability or even to know what they really are (16). For morningness-eveningness, the underlying distribution was modeled to have four thresholds to make it possible to have scores in each of the five

Table 1. Familial Polychoric Correlations for the Five Zygosity Groups, Between Spouses and Between Biological Parent and Offspring for the Morningness-Eveningness Score (1, 2, 3, 4, or 5)

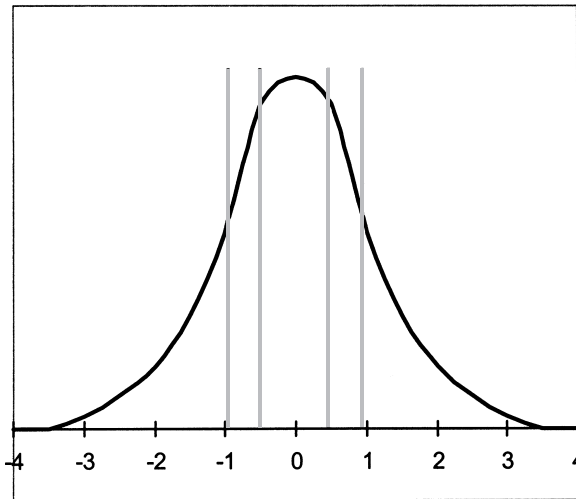|	Correlation	N^a		Correlation	N^a
MZM	0.382	277	Spouses	−0.116	1540
DZM	0.043	228	Father-son	0.031	1319
MZF	0.501	400	Father-daughter	0.117	1621
DZF	0.099	296	Mother-son	0.094	1447
DOS	−0.047	449	Mother-daughter	0.124	1792

[a]N was based on complete pairs; correlations were estimated including incomplete pairs also.

categories (Fig. 1). A score above a threshold makes the score fall in the next morningness-eveningness category. The four estimated thresholds were constrained to be the same for fathers, mothers, girls, and boys. Total variances were allowed to be different for these four groups, and the variance for the fathers was constrained as 1. This model specification is equivalent to standardizing all liability distributions to have unit variance and allow sex and generation differences in prevalence.

Correlations between twins and family members were calculated for the liability dimension (polychoric correlation). When the MZ correlations are more than twice the DZ correlations, this suggests that genetic effects do not operate in an additive manner. Different types of genetic effects can be considered in modeling the resemblance of family members. Sources of variation that were considered in modeling were additive genetic variation (A), dominance effects (D) and a random environmental deviation (E) that is not shared by family members. Their influence on the phenotype is given by parameters a, d, and e, which are equivalent to the standardized regression coefficients of the phenotype on A, D, and E, respectively.

The parameter estimates for the dominance variance were constrained to be half the estimates for additive genetic variance. Additive genetic effects occur when alleles at a locus and across loci "add up" to affect behavior. Dominance describes the nonadditive genetic effect in which alleles at a locus interact rather than add up to affect behavior. Because we do not have exactly the same combination of alleles as our parents (we inherit only one of each of their pair of alleles), we will differ from our parents for these nonadditive interactions. Therefore, parents and offspring do not share genetic variance due to dominance

Figure 1. Distribution of liability. Thresholds for morningness-eveningness were estimated as −0.940, −0.611, 0.461, and 0.894.

[r(Parent-Offspring) = 1/2 $r_g a^2$, where r_g is the genetic correlation between generations]. Siblings, like parents and their offspring, share half of the genetic variance that influences a character. However, siblings also share one-fourth of the dominance variance because full siblings can be expected to receive the same alleles from both parents one-fourth of the time and thus have the same dominance deviation. So, dizygotic twins share half of their additive genetic variance and one-fourth of the variance due to dominance (rDZ = 1/2 a^2 + 1/4 d^2). Monozygotic twins share all genetic variance because they are genetically identical (rMZ = a^2 + d^2). Figure 2 shows a graphical representation of the parent-twin model.

When data on adult twins are analyzed simultaneously with data from adolescent twins and parents, the path from parental A to offspring's A does not
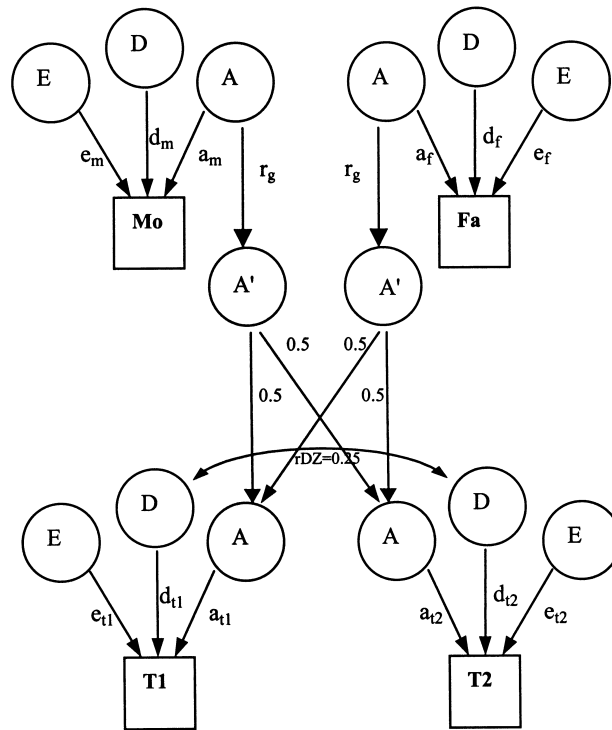
Figure 2. Path diagram of a parent-offspring model for dizygotic twins. Squares represent observed variables, and circles represent latent variables for fathers (fa), mothers (mo), and the twins (T1 and T2). A, D, and E represent additive genetic factors, dominance, and unique environment, respectively. The influence of A on the phenotype is given by the path coefficients a_m (for mothers), a_f (for fathers), a_{t1} (for twin 1), and a_{t2} (for twin 2). The influence of D on the phenotype is given by the path coefficients d_m (for mothers), d_f (for fathers), d_{t1} (for twin 1), and d_{t2} (for twin 2). The influence of E on the phenotype is given by the path coefficients e_m (for mothers), e_f (for fathers), e_{t1} (for twin 1), and e_{t2} (for twin 2). For same-sex twins, the path coefficients are equal ($a_{t1} = a_{t2}$, $d_{t1} = d_{t2}$, $e_{t1} = e_{t2}$). So, r(Parent-Offspring) = 1/2 $r_g a^2$, rDZ = 1/2 a^2 + 1/4 d^2, and rMZ = a^2 + d^2.

need to be fixed at 0.5, but can be estimated as $0.5*r_g$. In this way, the genetic correlation r_g between generations is estimated.

The effects of sex and generation were assessed by likelihood ratio tests by comparing the fit of a model with constrained parameter estimates to be equal across groups to one in which they were allowed to vary. Biometrical model fitting was done to raw data, making it possible to include data from incomplete families. Parameters a, d, and e and thresholds in the liability distribution were estimated using the computer program Mx (17). Twice the difference between the likelihood for the full model $-LL_0$ and that for a reduced model $-LL_1$ is distributed as a chi-square statistic [$\chi^2 = 2(LL_0 \times LL_1)$]. When this statistic was not significant compared to the difference in degrees of freedom df between the full and constrained models, the reduced model was accepted. The less-constrained model remained the preferred model if the difference was significant.

Eight different models were examined:

1. A full model in which estimates for a, d, and e were allowed to differ in magnitude between males and females and between parents and off-spring.
2. A reduced model in which the importance of dominance effects was explored by removing d from the model and examining the fit of the reduced model.
3. A reduced model in which the importance of all genetic effects was explored by removing a and d from the model and examining the fit of the reduced model.
4. A constrained model in which the parameter estimates for a, d, and e were constrained to be equal in magnitude for males and females to explore sex differences for the different factors.
5. A constrained model in which the parameter estimates for a, d, and e were constrained to be equal in magnitude across generations to explore generation differences for the different factors.

When a second data set was added consisting of adult twins of the same age as the parents in the first sample and analyzed simultaneously with the original data set; the following additional models were explored:

6. A full model in which estimates for a, d, and e were allowed to differ in magnitude between males and females and between parents and off-spring using extra data on adult twins to estimate the genetic correlation r_g between different generations.
7. A constrained model in which the genetic correlation was fixed at 0.5 to investigate whether different genes are operating in both generations.
8. A constrained model in which the parameter estimates for a, d, and e were constrained to be equal in magnitude for males and females to explore sex differences for the different factors.

RESULTS

Descriptive Statistics

Table 2 shows the number and percentage of subjects in every category of the morningness-eveningness scale for fathers, mothers, boys, and girls. For each group, the table shows that most subjects fall in the "neither" category. A Mann-Whitney test to investigate generation differences showed twins were significantly more evening oriented ($z = -14.558$, $P < .001$) than their parents. A Mann-Whitney test to investigate sex differences showed that women were significantly more morning oriented than men ($z = -5.843$, $P = .000$ in the first sample and $z = -3.431$, $P = .001$ in the second sample).

Table 1 summarizes the polychoric correlations for all zygosity groups and polychoric correlations between spouses and between parent and offspring. The correlations between monozygotic twins are more than twice the correlations between dizygotic twins. This suggests that genetic effects do not operate in an additive manner (dominance effects). However, the parent-offspring correlation is not much lower than correlations between dizygotic twins, which would be consistent with an additive genetic model.

The correlation between spouses is -0.12 and suggests that there is no assortative mating for morningness-eveningness in this study.

By fitting structural equation models to the data, generation differences between parents and offspring and differences between sexes were investigated. Table 3 shows the model-fitting results. The first model is a full model with an additive genetic factor A, dominance D, and a unique environmental factor E. The second model shows that removing dominance from the model decreases

Table 2. Number (N) and Percentage (%) of Subjects in Every Morningness-Eveningness Category for Fathers, Mothers, Boys, and Girls from the First Sample and for Men and Women of the Older Twin Sample

Morningness/ Eveningness	Father		Mother		Boys		Girls		Men		Women	
	N	%	N	%	N	%	N	%	N	%	N	%
1	304	19.4	527	30.5	148	9.6	241	12.4	28	20.3	63	33.3
2	153	9.8	183	10.6	114	7.4	192	9.9	9	6.5	13	6.9
3	681	43.5	609	35.3	634	41.2	738	38.1	59	42.8	66	34.9
4	167	10.7	187	10.8	247	16.0	326	16.8	16	11.6	13	6.9
5	261	16.7	220	12.7	397	25.8	440	22.7	26	18.8	19	10.1
Missing	308	—	148	—	110	—	104	—	11	—	18	—
Total	1874	100	1874	100	1650	100	2041	100	149	100	192	100

1 = morning active; 2 = moderately morning active; 3 = neither; 4 = moderately evening active; 5 = evening active.

Table 3. Model-Fitting Results Parent-Offspring Model

Model	−2 Log Likelihood	df	χ^2	P	Δdf
1. ADE sex and generation differences	20,001.613	6758			
2. AE sex and generation differences	20,008.672	6758	7.059	—	0
3. E sex and generation differences	20,128.705	6762	127.092	0	4
4. No sex differences in A, D, and E	**20,005.447**	**6762**	**3.834**	**.43**	**4**
5. No generation differences in ADE	20,024.399	6762	22.786	0	4

Goodness-of-fit indices were −2 log likelihood, degrees of freedom *df*, the computed chi-square statistic χ^2, the probability *P*, difference in degrees of freedom Δdf. Bold indicates best-fitting model.
A, additive genetic factor; D, dominance; E, unique environment.

the likelihood of the model (because V_D is constrained to be 0.5 V_A, there is no change in degrees of freedom). Removing both additive genetic factors and dominance from the model significantly worsened the fit of the third model. Constraining A, D, and E to be equal for both sexes did not significantly worsen the fit of the fourth model and shows that there are no sex differences in the magnitude of A, D, and E. In model 5, differences between parents and offspring were investigated by constraining A, D, and E to be equal among generations. This significantly worsened the fit of the model and shows that there are differences between parents and offspring. The best-fitting model (bold type in Table 3) of the data set is a full model with A, D, and E in which these factors are constrained to be equal across sexes. The standardized parameter estimates for this model are depicted in Table 4.

The model-fitting results shown in Table 5 were obtained by adding the second data set on morningness-eveningness of twins of approximately the same age as the parents of the original parent-offspring data set. The first model (model 6) is a full model with A, D, and E in which the genetic path between the generations is estimated as 0.15 and equals $0.5*r_g$. This implies that r_g is 0.30. In the next model (model 7), this correlation was constrained to be 0.5 ($r_g = 1$), and this significantly worsened the fit of the model. This suggests that different genes may be operating in both generations. In the last model (model 8), sex differences were investigated by constraining A, D, and E to be equal for both sexes. This did not significantly worsen the fit of the model, so the best-

Table 4. Standardized Parameter Estimates for the Best-Fitting Model

Subjects	Additive Genetic Factors	Dominance Effects	Unique Environmental Factors	Broad Heritability
Offspring	0.290	0.145	0.565	0.435
Parents	0.028	0.014	0.957	0.042

Table 5. Model-Fitting Results Generation Model (Including Second Sample)

Model	−2 Log Likelihood	df	χ^2	P	Δdf
6. ADE sex and generation differences, r free					
(0.15)	20,902.065	7073			
7. ADE sex and generation differences, r 0.5	20,915.733	7074	13.688	0	1
8. ADE no sex differences, r free	**20,906.456**	**7077**	**4.391**	**.22**	**3**

Goodness-of-fit indices were −2 log likelihood, degrees of freedom df, the computed chi-square statistic χ^2, the probability P, difference in degrees of freedom Δdf, genetic correlation between generations r.

A = additive genetic factor, D = dominance, E = unique environment.

fitting model (bold type in Table 5) was also a model without sex differences. Table 6 shows the standardized parameter estimates for the best-fitting model.

DISCUSSION

In this study, self-reported data on the morningness-eveningness of twins and their parents were analyzed. The question was addressed concerning the extent genetic and environmental factors contribute to individual differences in morningness-eveningness.

In several studies, differences have been found between men and women in their preference for morning or evening type. Our study investigated sex differences using data on twins and their parents. In both generations, women were significantly more morning oriented than men. This is in line with data of Motohasi et al. (18,19) and Park et al. (20), who found men's preference to be shifted more toward the evening than women. However, according to Steele et al. (21), men seem to be more morning oriented. Other studies failed to detect differences between men and women in morningness and eveningness (22,23). Assortative mating was investigated by examining the husband-wife correlation. Hur et al. (24) found a husband-wife correlation of 0.25 for morningness-eveningness ($n =$ 79), suggesting this could be the result of assortative mating. However, in our

Table 6. Standardized Parameter Estimates for the Best-Fitting Model, r_g = Free (0.15)

Subjects	Additive Genetic Factors	Dominance Effects	Unique Environmental Factors	Broad Heritability
Girls	0.293	0.147	0.560	0.440
Boys	0.293	0.147	0.560	0.440
Men	0.317	0.159	0.524	0.476
Women	0.317	0.159	0.524	0.476

study, the correlation between spouses is −0.12 ($n = 1429$), suggesting there is no assortative mating in this sample.

In general, people have been shown to display more eveningness in adolescence and young adulthood and then shift toward morningness with age. A study demonstrated a significant change toward a preference for eveningness over advancing grades in primary school and a junior high school in Japan (25). Carskadon et al. (26) evaluated sleep processes and circadian rhythm parameters in young humans, finding a relationship between adolescent development and circadian phase. Increasing age in adults was associated with higher morningness scores in other studies (8,21,22,27). In our study, parents were also significantly more morning oriented than their children. Ishihara et al. (22) suggested that this change might be due to a phase advance of circadian rhythm with increasing age. Duffy et al. (8) indeed found a difference between older and younger morning types, with older morning types waking at a significantly earlier circadian phase.

In our study, the percentages of the extreme types (1 and 5) are higher than those reported in the literature. Most investigators have used elaborated questionnaires in which questions were inserted to differentiate between present status of the individual and the real nature of the individual with regard to morningness-eveningness. In our study, the phenotypic pattern for morningness-eveningness was determined by quantifying the results obtained from one specific question offering five answer categories.

Using the parent-twin design, differences between sexes and generations in genetic and environmental factors influencing morningness-eveningness were investigated. No sex differences were found for the magnitude of genetic and environmental factors. The parameter estimates for genetic and environmental factors for the best-fitting model show that the importance of genetic factors is different for both generations. In twins (mean age 17 yr), genetic factors explained 44% of the variance, while for the parents (mean age 47 yr), genetic factors explained only 4% of the variance. In contrast, in the study of Hur et al. (24), genetic variability accounted for about 54% and age accounted for 3% of the total variance.

Different explanations are possible when relatives of different generations are less alike than relatives of the same generation. Genetic dominance effects can make children different from their parents because they do not have exactly the same combination of alleles as their parents. Thus, although genetic dominance may contribute to the phenotypes of parents and offspring, this genetic factor is not shared by them.

Alternative explanations for differences between generations are that genes are switched off with increasing age or that different genes are operating at different ages. In our study, genetic dominance effects can only explain a portion of the difference between generations. The difference in heritability can only be explained by the alternative possibilities. Using parent-twin data alone makes it impossible to disentangle these possibilities. The second series of analyses in-

cluded data from a small sample of adult twins (mean age 46.7 yr). Including these twins allowed us to estimate the genetic correlation between generations, which was estimated to be 0.30.

The correlation between generations turned out to be lower than 1, suggesting that different genes are expressed in both generations. Also, the parameter estimates for the older generation are comparable to the estimates found by Hur et al. (24) when the generation correlation is free (48% vs. 54%, respectively). This suggests that partly different genes that influence morningness-eveningness may be operating in both generations. Because the genetic correlation between the generations is more than 0, morningness-eveningness is also influenced by genes that are expressed in both generations.

When several genes affect morningness-eveningness, the alleles at different loci can add up to affect behavior, but they can also interact. This type of interaction between alleles at different loci is called *epistasis* (28). Any dominance deviations would inflate the sibling correlations, but not the parent-offspring correlation. Certain types of nonallelic interactions (epistasis), however, inflate the sibling and the parent-offspring correlations by an identical amount. The probability of detecting and identifying epistasis or separating it from dominance effects in humans is very small (29).

Since the sequence of the human genome has been identified, the search for genes will increase. Several candidate genes for circadian rhythm have been identified already: hPER1, hPER2, hPER3, HCRY2, hBMSL1, and hCLOCK. Possibly, these candidate genes are involved in morningness-eveningness and could contribute either to genetic differences between generations or to the genetic variance that remains stable over time (30–32).

ACKNOWLEDGMENT

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