Letters to the Editor

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Age at Menarche as a Fitness Trait: Further Considerations

To the Editor:

The extensive twin study of Treloar and Martin (1990) shows that most of the genetic variance of the age at menarche (AM) is nonadditive, typical of traits affecting genetic fitness. This is an important addition to our understanding of this major, but still elusive, physiological landmark. I wish to comment on their key assumption that earlier AM, on average, does in fact produce increased fertility.

First, it seems remarkable to me that there appear to be very few tests of this assumption, such as correlating AM with number of live-born children ever born (granting the problem of recall of AM in older women) or even with the age of the first-born child. The authors do not mention such tests. The only explicit test known to me is my own, and it supports the assumption, showing a very significant ($P < 10^{-5}$) *negative* regression, in Caucasians, of number of pregnancies (at about 26 years of age) on AM (see study mentioned below). I agree with Treloar and Martin that it is a strong inference that earlier AM, as an indicator of earlier sexual maturity, should result in increased fertility. This earlier maturity could have been especially important in our evolutionary past, before the Agricultural Revolution, when women (and men) often failed to survive to the end of their potential reproductive period.

Second, it may not be widely realized that earlier fertility can have a selective advantage in *two* ways. The obvious way is an increase in total fertility (number of live-born children ever born to a woman). But even if total fertility is unchanged, having children earlier still has a selective advantage. Consider two women who have completed their reproduction, each with a total of two live-born children. *A* had one child at age 20 years and one at age 25 years. *B* had each child two years earlier, at ages 18 and 23 years. *A*'s *annual* rate of production of children (and, proportionally, transmitted genes) is $1/20 + 1/25 = .090$; *B*'s *annual* rate is $1/18 + 1/23 = .099$, 10% larger. If this earlier production of children by *B* is due to genes causing earlier menarche, *B* would have a selective advantage; the "early menarche" genes would be selected for. This point, the selective advantage of having children earlier, has been made before in a different context: study of a gene (for Huntington disease) which changes the maternal age distribution (Reed 1959).

Third, in the hope that it may be replicated, I would like to draw attention to a result from a large biomedical study: tentative association of earlier AM with increased genetic heterozygosity. This finding, if true, would be relevant to the authors' consideration of the effects of inbreeding depression on AM. In a study of pregnant California women enrolled in a large maternal and child health study, I (Reed 1968) analyzed AM and five blood-group systems in 3,123 Caucasian women and 1,159 black women. A blood-group heterozygosity score—using the ABO, Rh (five antisera), MNSs, Kell, and Lu systems, where a score of 0 means no detectable heterozygosity, 1 means heterozygosity in one system, etc.—showed a formally significant ($P = \sim.0003$; but see below) *negative* correlation with AM in Caucasians, but not in blacks. Heterozygote selective advantage, of course, is one attractive mechanism for maintaining genetic polymorphisms such as blood groups. In spite of considerable effort by a num-

ber of investigators using large samples, however, selective differences which could maintain these and other worldwide polymorphisms (excepting sickle cell hemoglobin) have yet to be clearly demonstrated (reviewed in Reed 1975).

The above correlation, which is in the expected direction, unfortunately was from many significance tests, and the possibility that it was a type I statistical error could not be excluded. And because no one appears to have had a comparable body of data, this correlation has not, to my knowledge, been repeated. I believe that it should be.

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Age at Menarche and Fitness: Reply to Reed

To the Editor:

The twin data from the 1980-82 study (Treloar and Martin 1990) were not suitable for examining the relationship of age at menarche (AM) and fertility because few of the women had completed their reproductive years and many had barely entered them. However, we have now collected 8-year followup data on 3,676 of the 4,872 women in the original twin sample (which included 906 women from opposite-sex pairs). All women are now age ≥26 years, and 1,317 are age ≥45 and may be considered to have completed their reproductive years. Detailed data on reproduction have been collected, including age at first pregnancy (AFPREG) and total number of pregnancies (TOTPREG). Excluding women who have not been pregnant might obscure any relationship between AM and fertility, so in these cases we have recorded AFPREG as present age plus 1 year; nulliparous women age >46 years have been assigned AFPREG = 46. As Reed (1990) suggests above, we have also calculated number of children by age 26 years (NKID26) and average fertility (× 100) according to his formula (AVFERT). Since all women in the sample are aged ≥26 years, NKID26 has a valid value for everybody. The other three measures of fertility, however, are clearly age dependent in women who have not completed their childbearing years. For this reason, we examine relationships of AM and fertility separately in (*a*) women age <45 years and (*b*) women age ≥45. Since there is a slight correlation of AM itself with age (−.02 in those age <45 years, .05 in those age >45 years, and .08 overall), it may be important to adjust for age when examining relationships between AM and fertility.

While the fertility measures are all based on second-wave data (1988–90), AM is that reported in 1980–82, since this is nearer the event and likely to be more accurate (correlation of the two reports of AM 8 years apart is .80 in women age <45 years, .86 in women age >45 years, and .82 overall). In the full sample, correlations between AM and the four fertility measures are virtually zero (table 1). But this obscures interesting heterogeneity between patterns in the younger and older women. In women age <45 years, there are significant correlations with all four fertility measures in the direction expected if AM is associated with greater fertility, as found by Reed (1990) and as predicted by the direction of genetic nonadditivity (Treloar and Martin 1990). However, in the older women the correlations are all in the opposite direction, although smaller in size. We expect fitness relationships to be small, and the correlation coefficient is an insensitive way to explore slight relationships, especially if there is any nonlinearity. Therefore, we have examined the means of each fertility measure (adjusted for age) in three groups classified as to whether AM was early (age ≤11 years), average (age 12–13 years), or late (age ≥14 years). In the younger

Table 1

Fertility and AM Data

A. Relationship between AM and Four Fertility Measures

Group	r[a]	Fertility Measure,[b] for AM (in years) of			P[c]
		≤11	12–13	≥14	
		Age (in years) at First Pregnancy			
<45	.06	26.00	26.39	26.95	.021
≥45	−.04	28.85	27.30	27.65	.076
All	.02	27.01	26.73	27.20	.167
		Total No. of Pregnancies			
<45	−.06	2.13	1.98	1.83	.012
≥45	.05	2.84	3.33	3.18	.009
All	.01	2.43	2.48	2.29	.017
		No. of Children by Age 26 Years			
<45	−.06	.80	.75	.66	.081
≥45	−.02	1.08	1.15	1.09	.622
All	−.01	.91	.89	.81	.054
		Average Fertility (× 100)			
<45	−.06	6.20	6.09	5.50	.018
≥45	.04	9.25	10.27	9.99	.090
All	.01	7.41	7.62	7.01	.011

B. No. of Respondents

Group	No. of Respondents, for AM (in years) of		
	≤11	12–13	≥14
<45 (N=2,359)	347	1,362	650
≥45 (N=1,317)	204	623	490
Total (N=3,676	551	1,985	1,140

[a] Pearson correlations, Based on total numbers of respondents (i.e., N's shown in parentheses in last three rows).

[b] Data are means adjusted for regression on age.

[c] Probability of difference between three class means adjusted for regression on age.

women the linear trend to greater fertility with earlier AM is confirmed for all four variables, although not quite significantly for NKID26. In the older women it is those with average AM who appear most fit, although the differences are only significant for TOTPREG. This tendency toward stabilizing selection is also apparent in the total data—although for AVFERT those with late menarche are clearly at a disadvantage, and this may be true for NKID26 too.

We cannot easily reconcile the apparently different relationships between AM and fertility in the younger and older women in our sample. Results in the younger cohort may be influenced by incorrect allowance for age effects, particularly since these women have not completed childbearing. On the other hand, even though childbearing is complete in the older cohort, retrospective reports of AM may be less reliable. Greater fertility of women who are DZ twins (Martin et al. 1984) may also make it difficult to generalize to a nontwin sample. In any case, if AM has been an important fitness trait during human evolution, widespread use of contraception must lessen its impact now.

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Heterozygotes and Homozygotes: Discrimination by Chemical Cleavage of Mismatch

To the Editor:

The chemical reactivity, in a heteroduplex formed from wild-type and mutant DNA, of mismatched T and C bases with, respectively, osmium tetroxide and hydroxylamine is a useful tool for scanning mutations in the genome (Cotton et al. 1988). However, the definition of the homozygous or heterozygous state of the mutation is labor intensive and time consuming, being based on sequencing techniques. We report here a simple and efficient approach for identifying the status of homozygosity or heterozygosity for a specific mutation by using the chemical cleavage of mismatch (CCM) method.

Genomic DNA from two β-thalassemic patients and from a normal control was amplified using appropriate primers to obtain a 627-bp fragment encompassing exons 1 and 2 of the β-globin gene. By allele-specific oligonucleotide analysis, patient A was identified as homozygous for a nonsense C→T mutation at codon 39, and patient B was identified as a compound heterozygote for the same mutation and for a G→A substitution at position 110 of intron 1 (IVS I-110) (Kazazian and Bohem 1988). Both changes are identifiable by hydroxylamine reactions; in the case of codon 39 a C-A mismatch is formed in the heteroduplex by the sense strand of the probe, whereas in the case of IVS I-110 a C-A mismatch is originated by the antisense DNA strand. Internally labeled probes were prepared from DNA of both the patients and the normal control by adding 20 pmol of ^{32}P dCTP at the 25th cycle of PCR and performing 10 more cycles. The labeled PCR products were subsequently purified by means of PAGE.

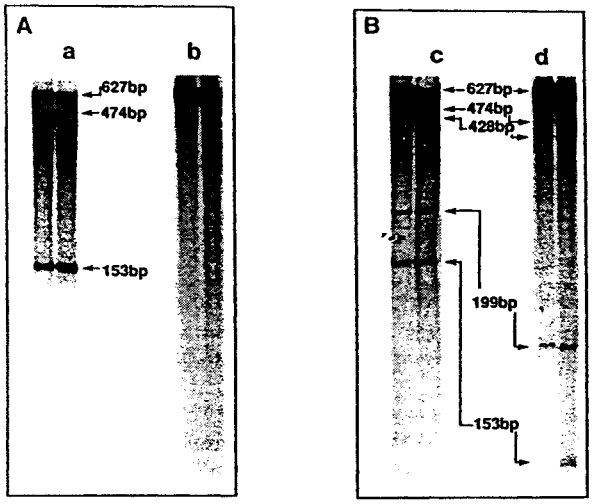

Figure 1 *A*, CCM analysis in patient homozygous for C→T transition at codon 39 of β-globin gene. The patient/control reaction (track a) shows the expected mismatch. As the original PCR product had been cleaved at the position of the mismatch, two cleavage products are found. The patient/patient reaction (track b) does not show any reactivity, as both alleles carry the same mutated sequence. *B*, CCM analysis in patient compound heterozygous for codon 39 mutation and for intronic G→A transition (IVS I-110). This patient carries a normal IVS I-110 allele and a mutant codon 39 allele on one chromosome, and on the other carries a mutant IVS I-110 allele and a normal codon 39 allele. Each mutation results in two cleavage products, so four bands are found in the autoradiograph when a probe with a normal sequence is used (track c). The presence of the normal sequences in the labeled probe obtained from the patient DNA results in the patient/patient reaction (track d) having a pattern identical to that of the control/patient (track c) and identifies the condition of heterozygote (as in the case of the control/patient reaction the two C-A mismatches are cleaved on opposite DNA strands).

Heteroduplexes were formed by annealing the labeled and the unlabeled DNA fragments according to a method described elsewhere (Cotton et al. 1988) and were reacted for 1-3 h with hydroxylamine. The unlabeled PCR fragment from each patient was first hybridized to a probe from a normal subject to detect the mismatches. Subsequent hybridization to a probe prepared from the patient himself allowed heterozygosity or homozygosity to be determined. The results are shown in figure 1. Hybridizing probe from patient A to DNA from patient A did not show any reactivity after CCM analysis (fig. 1, track b). This confirms that only one allele type is present and that the patient is