Table 1

Frequency Distribution of Size Differences between Microsatellite Alleles

No. of Repeats	Généthon	SSMM	MSMM
1	.812	.936	.810
2	.111	.047	.126
3	.036	.011	.037
4	.016	.004	.014
5	.011	.001	.007
6	.005	.001	.003
7	.003	.000	.002
8	.002	.000	.001
9	.001	.000	.001
10	.001	.000	.000

NOTE.—"No. of Repeats" indicates the difference in the number of dinucleotide repeats between adjacent alleles that have been ranked by their absolute size. SSMM = single-step mutation model. MSMM = multiple-step mutation model ($m = 2$), no mutational bias.

We have found that microsatellite allele frequency distributions tend to be positively skewed in favor of longer alleles, which is in agreement with an analysis of CAG repeats in the Huntington disease gene (Rubinsztein et al. 1994) and the unpublished results of W. Amos and D. Rubinsztein (cited in Rubinsztein et al. 1995). Our computer simulation results suggest that the underlying mutational model for generating new microsatellite alleles is likely to be asymmetrical and multistep. MSMM models with an absorbing boundary or with a mutational bias in favor of larger alleles can generate allele distributions that closely resemble those observed in the Généthon data. Models of directional evolution that result from mutational bias have been recently discussed (Rubinsztein et al. 1995; Primmer et al. 1996). Finally, the empirical mean frequencies of the ranked alleles derived from the Généthon analysis provide useful prior distributions to those applying Bayesian smoothing techniques (Lange 1997) to $(AC)_n$ microsatellite allele frequency estimates.

MARTIN FARRALL[1] AND DANIEL E. WEEKS[1,2]
[1]*Statistical Genetics Group, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford; and* [2]*Department of Human Genetics, University of Pittsburgh, Pittsburgh*

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Address for correspondence and reprints: Dr. Martin Farrall, Statistical Genetics group, Wellcome Trust Centre for Human Genetics, University of Oxford, Windmill Road, Oxford, OX3 7BN, United Kingdom. E-mail: mfarrall@well.ox.ac.uk

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Some Underlooked Properties of the Multifactorial/ Threshold Model

To the Editor:

Some years ago, when the multifactorial/threshold (MFT) model was beginning to be recognized (by some) as a useful way of thinking about the causes of common congenital malformations, I noted one of its implications. "It follows from the MFT model that in conditions appearing more often in one sex than the other, the sex ratio should change as the frequency changes" (Fraser 1971, p. 90). I suggested that such changes in the sex

ratio of a condition might be a more sensitive indicator of changes in its frequency than direct counts would be. Since then, there have been several relevant examples of changes in sex ratio with differences in frequency of a trait. These have evoked a number of explanations that, though ingenious, may be unnecessary; the answer may lie in the above-mentioned property of the MFT model.

The MFT model postulates a continuous distribution of "liability" to a particular defect and a threshold separating the continuous distribution into discontinuous parts, with only those individuals falling beyond the threshold having the defect. Cleft palate in the mouse is a classic example, with substantial experimental support (Fraser 1976). In this case, liability is a reflection of the developmental stage at which the palate shelves move toward closure. The threshold is the latest stage at which closure is still possible. Embryos in which shelf movement occurs later than this have cleft palate. Thus, the embryos with later closure are more susceptible, or liable (Fraser 1980*a*).

A number of predictions can be made from the MFT model, relating recurrence risk to sex of proband, severity of defect, number of affected relatives, degree of relationship to proband, and population frequency of the trait. These have been discussed extensively elsewhere (Fraser 1976, 1980*b*). Note that all these properties relate to how the frequency of a trait changes as the position of the liability distribution changes relative to the threshold and not to whether the distribution is normal (polygenic) or multimodal (several genes with low penetrance).

Two other predictions of the MFT model, to be discussed in this article, have been largely overlooked. The first of these addresses how changes in the sex ratio of a MFT trait can reveal changes in liability. For instance, the American Society for Human Genetics Statement on Behavioral Genetics (Sherman et al. 1997) uses emotional stability as an example of how sex-ratio differences are exaggerated at the tails of the frequency distribution in conditions where the distribution differs in the two sexes. For this multifactorial trait, males have a somewhat higher mean emotional stability score than females, but at the upper 1% of the distribution there is a striking excess of males. Figure 1 illustrates why. The dashed curve represents the distribution of "liability to stability" scores for males, and the solid curve that for females. The (arbitrary) threshold, T2, demarcates the upper 1% of the population, who could be considered those with the "ultrastability" trait. Note that in this group there is an obvious excess of males; that is, there are many more males than females in the area under the curve beyond T2. If the threshold is set at (say) the 5% level (T1), there will be an increase in frequency of the trait, but the excess of males beyond the T1 level is not as great as it was beyond the T2 threshold (i.e., the proportion of "ultrastable" males is smaller). Thus, the sex ratio of "ultrastable" individuals shifts toward equality as the frequency of the trait (number of individuals beyond the threshold) increases.

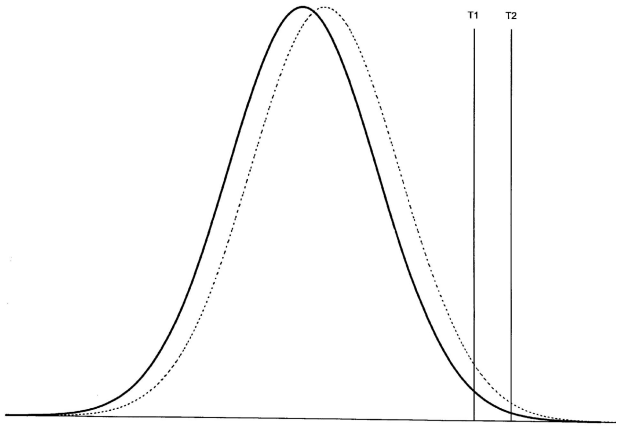

Figure 1 Multifactorial threshold model for a condition in which males and females have different frequencies. For details, see text .

For congenital malformations, one cannot see the distribution of liability for the trait but only the proportion of individuals who fall beyond the threshold (the frequency of the trait). In cleft lip, with or without cleft palate (CLP), for example, more males than females are affected, so we infer that the liability distribution for males is farther to the right than for females. Figure 1 now represents the distributions of liability to CLP for males (*dashed line*) and females (*solid line*); the threshold separates those with the trait (in the tail of the distribution) from those without it. Again, if the distribution of liability shifts to the right, so that the frequency increases, the sex ratio shifts toward equality. (For simplicity's sake, fig. 1 illustrates this by shifting the thresholds to the left, rather than the distributions to the right).

CLP provides several examples of this effect. For example, one would expect liability to be lower in families with one affected individual (simplex) than in those with more than one (multiplex), so the sex ratio should be closer to 1 in the multiplex families—and it is. The sex ratio in simplex versus multiplex families is reported as 2.0 versus 1.5 by Fraser (1980*b*) and as 3.2 versus 1.4 by Ray et al. (1993). An explanation is suggested by Ray et al. Perhaps "the loading of environmental factors predisposing to CLP is higher in multiplex families and results in a higher frequency of the less susceptible sex (females) becoming affected" (Ray et al. 1993, p. 1010). Possibly, but the simplest explanation is that the sex ratio shift is just what is expected for an MFT trait, with different liability distributions for the two sexes.

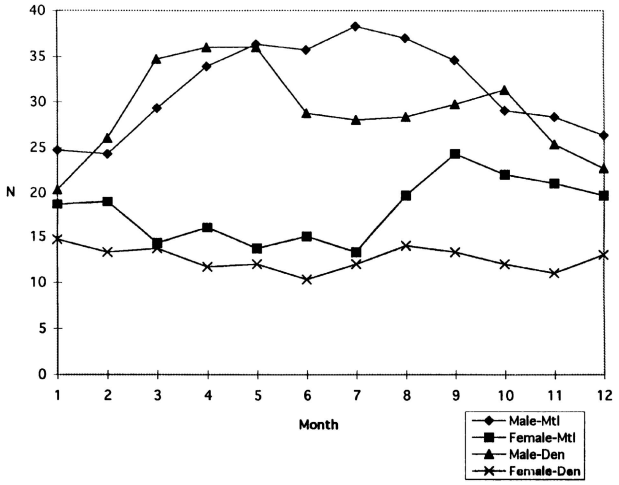

Figure 2 Month of birth for children with CLP, by sex, for Montreal and Denmark. For details, see text. The top two graphs represent males.

One might also expect that the liability distribution of CLP would be farther to the right in cases with associated malformations than in "isolated" cases, since, for one thing, this group would include some cases with syndromes in which CLP is a recognized component. For nonsyndromic, or "isolated," cases of cleft lip, the liability distribution would cross the threshold at a point where ~1 case in 1,000 would fall beyond the threshold, this being the frequency of CLP. For "syndromic" CLP (with reduced penetrance for CLP, of course) the proportion of patients with the syndrome who have CLP would be much higher than this, or else CLP would not be recognized as part of the syndrome. For cases in which the associated malformations were both caused by a teratogen, the liability to CLP in exposed embryos would also presumably be higher than in the general population. Thus, cases with associated malformations should have a liability distribution farther to right, and therefore a lower sex ratio, than those without them. This has been reported by Källen and Harris (1996), who found a sex ratio of 1.3 for those with versus 1.9 for those without associated malformations. The authors suggest that mutants with associated malformations consist of two subgroups: one equivalent to the children with isolated facial clefts and another with a normal sex ratio. This is a possibility, but one does not need another explanation; the MFT model predicts it.

This phenomenon is not limited to CLP. Lubinsky (1997), in an interesting article on sex-biased anomalies, points out that the sex ratio shifts toward equality in patients with versus those without additional malformations in the case of anencephaly, spina bifida, encephalocele, single umbilical artery, and diaphragmatic hernia. He postulates that these malformations may have complex and heterogeneous origins and that "with dual origins 'strong' events could increase the involvement of otherwise minor second processes with opposite bias from the primary disturbance" (p. 227). This is ingenious, but would it escape Occam's razor? The MFT model provides a simpler explanation.

A final example comes from my own experience. The gene for the autosomal dominant Van der Woude, or lip-pit syndrome, produces CLP in some carriers and isolated cleft palate (CP) in others. I thought it would be interesting to see if the cases of CLP and CP in families with this syndrome showed the same excess of males in CLP and females in CP patients that they do in nonsyndromic patients. To my surprise, they did not; both sex ratios shifted toward equality. "But of course, stupid," I said to myself. Both malformations have a higher penetrance in the syndrome than they do in isolated cases, so the shift in sex ratio toward equality is just what one would expect.

Another overlooked prediction from the MFT model, for traits with sex ratios deviating from 1, is that epidemiological variations are more manifest in the sex with the higher frequency. Observe in figure 1 that as the distribution is moved to the right (or the threshold to the left) and the frequency increases, the slope of the curve at the threshold increases. It is much steeper at T1 than at T2, but more so for males (dotted curve) than for females. This means that a change in liability for a rare trait, where the threshold is near the tail of the distribution, should result in a smaller change in frequency than it would for a more frequent trait, where the threshold is farther to the left relative to the distribution. Thus, anything altering liability would lead to a greater change in frequency in the sex with the higher frequency. So, in a search for factors that alter liability, and thus change the frequency, any change would be more discernible in the sex with the greatest frequency. Thus, one would expect the variation in frequency with various epidemiological factors to be more apparent in females in the case of anencephaly (which is more frequent in females), and more apparent in males in the case of CLP.

Since neural-tube defects show variations with season of birth, which could be in part a result of seasonal variations in maternal vitamin intake, I wondered whether CLP would also show an association with season of birth, since there is growing evidence that liability to CLP varies with maternal vitamin intake (Tolarova and Harris 1995; Czeizel et al. 1996; Shaw et al. 1997). For the above reasons, such an effect should be more evident in males. Previous studies of variation in season of birth for CLP, with sexes combined, have been inconsistent. A review of cases of CLP seen at the Montreal

Children's Hospital from 1950 to 1996 does indeed show a significant variation in males (fig. 2), with a peak around July–August, which is not present in the females (Fraser and Gwynn 1998). Figure 2 also shows data on season of birth from Fogh-Andersen's classic monograph on CLP (1942), which we have analyzed by sex. There is a significant variation in males, with a peak in April–May but not in females (F. C. Fraser and X. N. Rahnema, unpublished data). A similar peak for CLP (sexes combined) is reported from Finland (Rintala 1983), and it would be interesting to see whether this variation was more evident in males in this and other populations. The reasons for these variations remain to be clarified. There is some preliminary evidence that the effect may have diminished or disappeared in recent years. But, whatever the explanation, the point here is that for threshold traits that are more frequent in one sex than the other, epidemiological variations may be revealed more effectively by examining the sexes separately.

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F. Clarke Fraser
Departments of Human Genetics and Biology, McGill University, and the F. Clarke Fraser Clinical Genetics Unit, The Montreal Children's Hospital, Montreal

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Address for correspondence and reprints: Prof. F. Clarke Fraser, Department of Human Genetics, McGill University, 1205 Ave. Dr. Penfield, Montreal, Quebec H3A 1B1, Canada. E-mail: clarke_fraser@maclan.mcgill.ca

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