Menstrual cycle alters face preference

Women prefer slightly feminized male facial shapes[1]. Such faces (Fig. 1a) are given positive personality attributions[1] that might correlate with actual behaviour[2]. In contrast, masculine features seem to signal immunological competence[3]. Heritable benefits can be realized only if conception follows copulation, so women might be more attentive to phenotypic markers indicating immunological competence during the follicular phase of the menstrual cycle when conception is most likely[4,5]. Consistent with this hypothesis is the observation that women's preference for the odour of men with low fluctuating asymmetry (a correlate of testosterone-facilitated trait size and developmental stability) increases with the probability of conception across the menstrual cycle[5]. Symmetrical men report more extra-pair copulation partners[6], and extra-pair copulation rates peak in mid-cycle[7]. Here we show that female preference for secondary sexual traits in male face shapes varies with the probability of conception across the menstrual cycle.

Japanese subjects reporting regular menstrual cycles and no use of oral contraceptives ($n=39$, mean age 21 years, mean cycle length 30 days) answered questions about the average cycle length and date of onset of previous menses. From this information, two testing sessions were arranged for the sampling of individuals' preferences during phases of both high and low conception risk in their menstrual cycles. Ovulation was assumed to occur 14 days before the onset of menses. Sessions after ovulation and before the onset of menses (the luteal phase) and sessions during menses were classified as 'low conception risk'. Sessions in the follicular phase (between the end of menses and ovulation) were classified as 'high conception risk'[4,5,7]. Subjects were asked to select the face that they considered most 'physically attractive' ('miryoku-teki') from five Caucasian and, separately, five Japanese male faces (40% and 20% feminized, on average, and 20% and 40% masculinized). Subjects were also asked whether they currently had a 'steady boyfriend' ('tsukiatteiru'). Stimuli were symmetrical versions of those used (and illustrated) in previous research[1] but with hair visible, presented with full counterbalancing.

Repeated-measures analysis of variance (ANOVA) showed a significant main effect of conception risk (variance ratio $(F_{(1,37)})=9.47$; $P<0.004$), with subjects preferring faces that were less feminized in the high-conception-risk phase than in the low-conception-risk phase. No effects of stimuli origin (Caucasian or Japanese; Fig. 1b), partner or any interactions were found, although there were trends indicating that women with a partner preferred faces that were more masculine ($F_{(1,37)}=3.59$, $P=0.066$) and underwent a greater cyclic change in preference than those without partners ($F_{(1,37)}=3.2$, $P=0.08$).

In a second experiment, an interactive method[1] was used that allowed British subjects (mean age 20 years) to alter composite male face shapes along continua ranging from 50% feminized to 50% masculinized. Subjects were asked to choose the most attractive face for a 'long-term relationship' or a 'short-term sexual relationship'. Five facial continua (two from the experiment above[1] and three new ones; Fig. 1a) were prepared from separate symmetrical composites. Cycle and contraception details were obtained from 65 subjects, who completed three or four sessions at approximately weekly intervals: 27 made long-term and 28 made short-term judgements in all sessions. A further ten subjects made both judgements. Responses to faces were averaged over 'low-risk' and over 'high-risk' sessions.

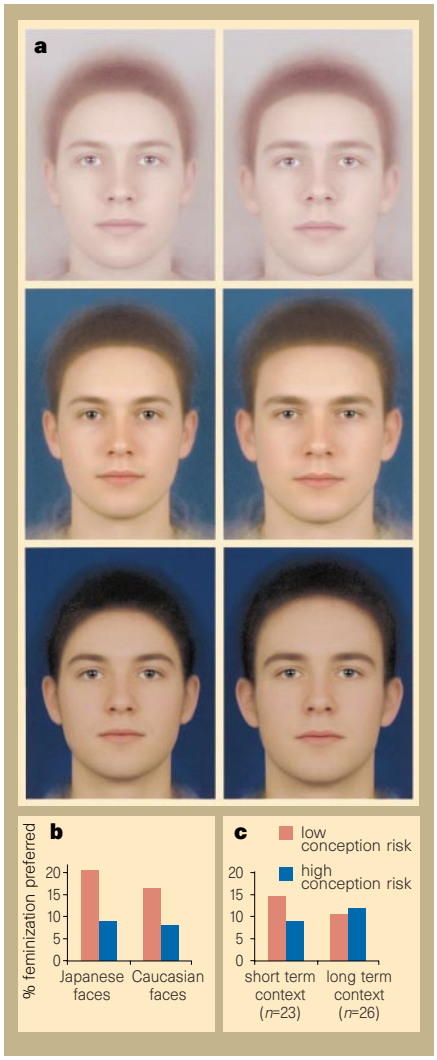

Figure 1 Cyclic shifts in the degree of femininity preferred in male faces. **a,** Face shapes that are 50% feminized (left) and 50% masculinized (right). Top: constructed[1] from 26 males, mean age 19.7 years; 37 females, 18.7 years. Centre: 21 males, 21.0 years; 40 females, 21.0 years. Bottom: 18 males, 19.8 years; 38 females, 20.8 years. **b,** Mean feminization preferred in Japanese and Caucasian composites by Japanese subjects ($n=39$) in high- and low-conception-risk phases. **c,** Mean femininity preferred across faces for short- and long-term conditions (experiment 2) in high-risk and low-risk phases.

Repeated-measures ANOVA for subjects not using oral contraception ('short-term', $n=17$; 'long-term', $n=20$; both judgements, $n=6$) showed no main effect of conception risk ($F_{(1,47)}=2.13$, $P=0.151$). However, conception risk interacted with type of relationship (short-term or long-term; $F_{(1,47)}=5.39$, $P=0.025$). For a short-term sexual relationship, the preferred face shape was less feminine during the high-conception-risk phase, whereas preferences remained constant when women judged attractiveness for a long-term relationship (Fig. 1c). Subjects preferred different levels of femininity in different composites ($F_{(4,188)}=11.1$, $P<0.01$), but the lack of significant interaction (risk × stimuli × relationship, $F_{(4,188)}=0.79$, $P=0.53$) showed that the cyclic change in preference was present for all stimuli. An analysis of data from subjects that were using oral contraception ($n=22$) revealed no cyclic changes in face shape preference ($F_{(4,96)}=0.001$, $P=0.97$) or interactions.

Dominance and quality as a parent are attributions made at opposite ends of the continuum relating to facial masculinity[1], and each might be associated with costs and benefits to reproductive success. A preference for males with a more masculine appearance might confer benefits for offspring in terms of resistance to disease but confer costs due to potentially decreased paternal investment.

In humans, concealed ovulation and limited visual similarity between offspring and their fathers[8] can result in uncertainty of paternity. Such uncertainty, coupled with converging evidence for cyclic changes in female sexual behaviour and preferences for male characteristics[5,7,9,10], suggests that female mating strategy need not be entirely exclusive. As in some other species[11], selection might have favoured human females who pursued a mixed mating strategy under some ecological and social conditions. Women with a long-term sexual partner are more likely to have extra-pair copulations in the follicular phase of the cycle than during the luteal phase or menses[7]. A female might choose a primary

partner whose low masculine appearance suggests cooperation in parental care ('long-term' preferences are unchanged across the menstrual cycle) but occasionally copulate with a male with a more masculine appearance (indicating good immunocompetence) when conception is most likely. Sexual behaviour arising from cyclic preferences might allow individuals to accrue benefits from polyandry while maintaining the advantage of ostensive monandry.

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Limbs move beyond the Radical fringe

The *fringe* genes and the Notch signalling pathway are important in limb development in vertebrates and in *Drosophila*, helping to establish the 'organizing centres' that are required for the proximal–distal outgrowth of appendages[1–5]. Three vertebrate Fringe genes have been identified: *Manic* (*Mfng*), *Lunatic* (*Lfng*) and *Radical fringe* (*Rfng*)[4–8]. Here we show that the mouse *Rfng* gene is not required for limb development, even though it is expressed in the developing limb bud. But we have found that several developmental defects, which at first we attributed to a loss of *Rfng* function after mutating the gene, in fact arose as a result of the insertion of a selection cassette into the *Rfng* locus that affects the expression of a neighbouring gene or genes. Our findings highlight the need for optimization in designing mutant alleles to probe developmental processes[9,10].

Studies of *Rfng* expression in chick limb mutants and gain-of-function experiments in limb buds using retroviral misexpression both indicated that the juxtaposition of cells that did and did not express *Rfng* is sufficient for the formation of the apical ectodermal ridge (AER), an organizing centre that forms at the boundary between dorsal and ventral cells at the distal edge of the limb bud[4,5]. We have used homologous recombination in mouse embryonic stem cells to investigate *Rfng* expression in the mouse limb bud and to test whether it is necessary for the formation of the AER.

We inserted the *Escherichia coli lacZ* gene into the *Rfng* genomic locus to create the $Rfng^{lacZKI}$ 'knock-in' allele. Analysis of β-galactosidase activity in $Rfng^{lacZKI}$ limb buds revealed expression in the developing and mature AER (Fig. 1a–c). To create a null allele, $Rfng^{\Delta 1\text{-}8neo}$, we deleted 2.4 kilobases of the *Rfng* genomic locus containing the entire coding region (exons 1–8) and replaced it with a 'PGKneo-selection' cassette (which confers a resistance to the antibiotic neomycin and acts as a means for selecting these clones) in the opposite transcriptional orientation.

No homozygous $Rfng^{\Delta 1\text{-}8neo}$-deficient mouse survived past birth ($n = 244$ progeny from heterozygote intercrosses on a mixed 129P2 × C57BL/6J hybrid background; $n = 70$ progeny from heterozygote intercrosses on a mixed 129P2 × 129S6 hybrid background). The mutant mice displayed several developmental defects, including a cleft palate and reduced mandible (64%), exencephaly (24%), and forelimb defects (12%) consisting of soft-tissue syndactyly or oligodactyly (total $n = 225$ on a mixed 129P2/OlaHsd × C57BL/6J hybrid background, examined between 18 days postcoitum (d.p.c.) and birth; Fig. 1).

We went on to remove the PGKneo-selection cassette to create the $Rfng^{\Delta 1\text{-}8}$ null allele by two independent methods: mating $Rfng^{\Delta 1\text{-}8neo}$ mice to mice that expressed Cre

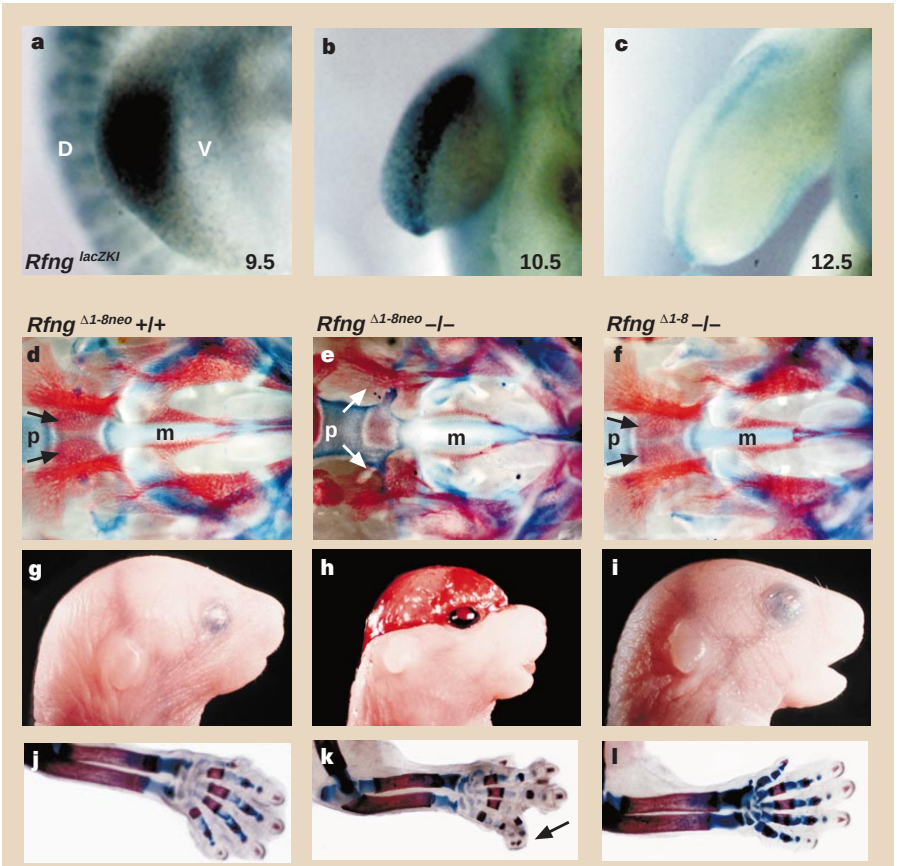

Figure 1 *Rfng* is expressed in the mouse limb bud and is not required for limb development. *Rfng* expression in $Rfng^{lacZKI}$ heterozygote limbs, as shown by X-gal staining, at: **a,** 9.5 d.p.c.; **b,** 10.5 d.p.c.; and **c,** 12.5 d.p.c. Dorsal (D), left; ventral (V), right. Replacement of the *Rfng* locus with a PGKneo-selection cassette results in pleiotropic developmental defects. **d, g, j,** Wild-type fetuses; **e,h, k,** $Rfng^{\Delta 1\text{-}8neo}$ homozygotes; and **f, i, l,** $Rfng^{\Delta 1\text{-}8}$ homozygotes at 18.5 d.p.c. **d–f,** Skeletal staining of the secondary palate. Anterior is to the right. **d,** The palatal (p; indicated by arrows) and maxillary (m) shelves are nearly fused. **e,** $Rfng^{\Delta 1\text{-}8neo}$ homozygote palatal shelves (p; white arrows) have not elevated towards the midline, and the maxillary shelves (m) are wider than in the wild type. **f,** $Rfng^{\Delta 1\text{-}8}$ homozygote, showing normal palatal (p) and maxillary (m) shelf positioning. **g,** Wild-type fetus with closed cranium. **h,** $Rfng^{\Delta 1\text{-}8neo}$ homozygote, showing exencephaly. **i,** $Rfng^{\Delta 1\text{-}8}$ homozygotes are indistinguishable from wild type (**g**). **j–l,** Skeletal staining of the forelimbs. Anterior is up, posterior is down. **j,** $Rfng^{\Delta 1\text{-}8neo}$ wild-type limb has five digits. **k,** $Rfng^{\Delta 1\text{-}8neo}$ homozygote, showing soft-tissue syndactylism of digits 4 and 5 (arrow). **l,** $Rfng^{\Delta 1\text{-}8}$ homozygotes are indistinguishable from wild type (**j**).