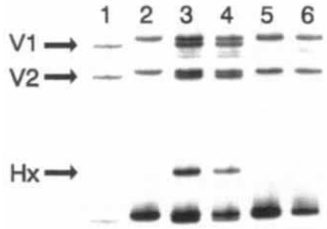

FIG. 2 Mutation analysis. Single-strand conformation polymorphism analysis of exon 11 of the *BRCA1* gene. Lane 1 contains the PCR product from the arrowed individual from pedigree 475, lane 3 is from her father and lane 4 from her mother. Lanes 2, 5 and 6 are normal controls. The two variant bands V1 and V2, and the heteroduplex band Hx, are arrowed. Methods are available from the authors.

As no wild-type sequence was present, we tested whether this individual was homozygous for this mutation, or was heterozygous for the AA_{2800} and had a deletion or polymorphism preventing amplification of the other allele. We screened lymphocyte DNA from her father and tumour DNA from her mother for the AA_{2800} mutation. Both her parents were heterozygotes for the AA_{2800} mutation, indicating that she is an AA_{2800} homozygote. We have also carried out haplotype analysis on this family and have found that the AA_{2800} homozygote is homozygous for five *BRCA1* flanking markers from *THRA1* to D17S579 (data not shown).

This woman is the first example, to our knowledge, of a human homozygote for a high-penetrance cancer susceptibility gene. Her cells should be invaluable in studying the function of the BRCA1 protein. It appears that her risk of cancer may be similar to that of heterozygotes for the AA_{2800} mutation, as she had breast cancer at age 32. The existence of viable *BRCA1* homozygotes has ramifications for the screening of high-risk women, as it is possible that a parent who is found to be heterozygous for a clinically meaningful mutation may have a second, unidentified alteration in the other *BRCA1* allele. The daughter of this parent could then be misdiagnosed as not having inherited a high-risk allele.

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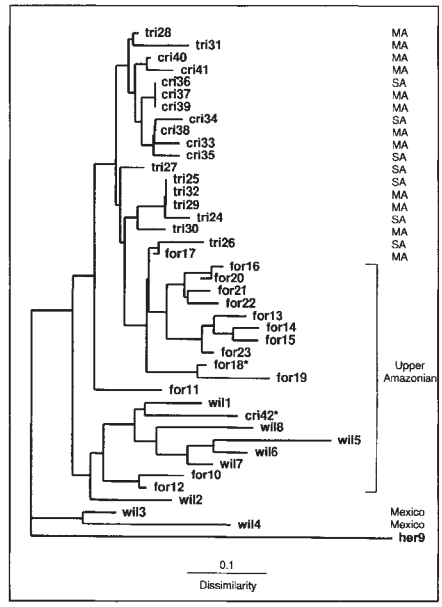

Clustering of cacao accessions based on genetic dissimilarity and the neighbour-joining method. Illustrated is an unrooted, strict consensus tree of 6 tied trees (wil, wild; cri, Criollo; for, Forastero; tri, Trinitario). Right-hand side, geographical origin of accessions (MA , Mesoamerica; SA, South America). Two accessions, for18 and cri42, are in the Upper Amazonian cluster, yet their geographical origins are Mexico and Trinidad, respectively. The 'wild' accessions from Mexico are located at the base of the tree (wil3 and wil4).

Origins of cacao cultivation

SIR — The origin and early domestication of cacao (*Theobroma cacao* ssp. *cacao* L.) remains an elusive issue as a result of uncertainty about the historical distribution of wild populations, ancient cultivations, human-mediated dispersion and continued crossing between cultivars and wild populations. Hypotheses on cacao origins either envisage independent domestication of the Criollo cultivar in Mesoamerica (from the ssp. *cacao*) and the Forastero cultivar in South America (from the ssp. *sphaerocarpum*)[1] or place the site of domestication of both cultivars in South America[2]. Neither hypothesis has strong support from morphology or ecology. Historical evidence shows that cacao was grown in the Yucatan peninsula in 1566, when an early chronicler wrote: "They have sacred groves where they cultivated certain trees, like cacao"[3]. Discovery of plants similar to wild cacao from the Lacandon forest (*T. cacao* ssp. *cacao* f. *lacandonense* Cuatrecasas) in 'sacred groves'[4] in Yucatan and additional collections from the Lacandon forest support a Mesoamerican origin for the Criollo variety, although recent genetic evidence supports an Amazonian centre of origin for both varieties[5,6]. Using molecular markers to characterize new accessions, we now show that Mexican 'wild' collections are genetically distinct from the South American wild plants and modern cultivars. Recently found 'wild' Yucatan plants in Maya 'sacred groves' are most probably the closest living representatives of an ancient Maya cultivar.

Molecular markers are useful for determining relationships among wild and domesticated plants, especially random amplified polymorphic DNA (RAPD) markers in cacao[5–7]. We surveyed 'wild' cacao populations from Mexico, wild populations from the Upper Amazonian region, three cultivars (Criollo, Forastero, Trinitario), and the sister genus *Herrania*. We tested 60 decamer primers, of which 24 produced consistent, robust amplification products resulting in a total of 105 loci across all accessions[8]. Relationships among accessions were accessed by neighbour-joining[9] on genetic dissimilarity (see figure). The analysis places the Mexican 'wild' collections at the base of the tree. Short branch lengths indicate a high degree of similarity among cultivars. Average dissimilarities between cultivars (see table) reveal fewer differences between Criollo and South American wild accessions than between Criollo and Mexican wild accessions. The basal position of Mexican 'wilds' and the greater similarity among South American wilds and all cultivars support the South American origin of present-day cultivated cacaos.

Our results indicate that Criollo cultivars are more similar genetically to South American germ plasms than to Mesoamerican 'wilds'. Other studies have

GENETIC DISSIMILARITIES BETWEEN GROUPS OF CACAO ACCESSIONS

Group	Mean dissimilarity	s.e.m.
Mexican 'wilds' × S. Amer. wilds	0.111	0.006
Mexican 'wilds' × Forastero	0.111	0.005
Mexican 'wilds' × Criollo	0.104	0.008
S. Amer. wilds × Forastero	0.101	0.003
S. Amer. wilds × Criollo	0.082	0.003

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confirmed the close relationship of the Criollo and Forastero cultivars and South American wilds[5,6]. These data imply that cacao cultivations of the Maya do not exist in modern germ-plasm collections. Managed groves were probably abandoned after European colonization, with a few remaining in isolation, protected by the Maya descendants. Rediscovered populations in Mexico are either derivatives of previously undetected wild cacao or remnants of ancient cultivars of the Maya people. The occurrence of the Yucatan 'wild' accession in known Maya 'sacred groves' supports the latter suggestion.

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Tuatara sex determination

SIR — Temperature-dependent sex determination (TSD) exists in three orders of living reptiles[1–3]. Here we report evidence that both surviving species of a fourth order (Sphenodontida) also exhibit this phenomenon. This finding is important to the conservation of sphenodontidans, and increases confidence in the suggestion that TSD is the ancestral sex-determining mechanism in reptiles[4].

We suspected that the lizard-like reptile tuatara (*Sphenodon punctatus*) exhibited TSD after observing total sex biases among locatable offspring for three zoo-incubated clutches. We subsequently sexed juvenile *S. punctatus* remaining in New Zealand from experiments in 1986 in which wild eggs were incubated under controlled conditions[5,6]. Live offspring were sexed by laparoscopy and available carcasses by dissection and/or histology. Incubation temperature had a highly significant effect on sex (P=0.001). Sex ratios (F:M) were 9:0 at 18 °C, 31:3 at 20 °C and 4:13 at 22 °C, temperatures within the range experienced by natural nests[7]. Although 32.4% of eggs failed and were unsexed, differential mortality of sexes pre-hatching is, as in other reptiles[8], unlikely to explain the results. At 20 °C, for instance, 83/115 eggs hatched and among 34 that hatched 91.2% were female. If we assume this sex ratio for all 83 hatchlings and also assume the extreme case of all dead embryos being male, the calculated sex ratio (76 F: 39 M) is still significantly biased (P<0.001). Differential mortality post-hatching is unlikely, as sex ratios at 20 °C show a female bias both in juveniles sexed live (23:2; P<0.001) and in those sexed dead (8:1; P = 0.04).

Data from an egg-incubation programme generating *S. guntheri* for reintroduction to the wild[9] also suggest that TSD exists. Wild eggs were incubated half-buried at −430 kPa and at 18 or 22 °C or a variable temperature regime (mean temperature 19.8–20.6 °C; temperature varied gradually over 18→23→18 °C during incubation). Overall hatching success was 88.0%. Sex ratios (F:M) among dead juveniles (live offspring were too small for laparoscopy) were 17:0 (18 °C, P<0.001), 3:0 (22 °C, P = 0.250) and 0:7 (variable, P<0.02). The male bias under the variable regime may result from temperatures >22 °C for at least 2 weeks in the middle of incubation, which in other reptiles is within the temperature-sensitive period[1]. Both species exhibited 100% females at 18 °C and a tendency towards males under a warmer regime(s). These observations fit the type Ib[2] or FM[1,10] pattern of TSD in reptiles, though further studies are necessary to rule out a type II (FMF) pattern[3].

In rare sea turtles, some incubation practices performed without knowledge of TSD have apparently resulted in male-biased sex ratios of reduced benefit to conservation[11,12]. Tuatara are restricted to 30 offshore islands of New Zealand[13]. Fortunately, the range of temperatures used in incubation programmes for both species has resulted in an estimated 68–77% of offspring being female, a favourable ratio for conservation purposes.

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Mouse knockouts rule OK

SIR — The value of genetic analysis to the study of learning and memory, specifically the value of mouse null mutants, has recently been questioned by A. Routtenberg in Scientific Correspondence (*Nature* **374**, 314–315; 1995). The objection was raised that mice harbouring null mutations are "reactionisms" (*sic*): another gene product, or system of gene products, may compensate for the missing gene product. As a consequence, data generated in the analysis of null mutants may be uninterpretable, presumably because of the complex interactions among the many genes involved in learning and memory.

The objection is based on the ill-founded assumption that an inability to observe a phenotype must mean that there is no phenotype to observe. But the maintenance of a gene is *prima facie* evidence that its absence would occasion a decrease in fitness (a phenotype), though not necessarily one easily observed under limited laboratory conditions. This mistake was compounded by overstating the significance of the synergism between mutations that frequently produces new phenotypes: this is not a novelty of mouse genetics, but a common occurrence even in *Escherichia coli*.

Complex biological processes will have complex genetics. This truism can easily be illustrated by considering mutations in *E. coli*: mutations affecting the catabolism of lactose define a relatively simple genetic pathway, whereas those affecting the doubling time of *E. coli* on rich medium would define an extremely complicated genetic system. Only in such simple pathway systems as intermediary metabolism can we expect simple genetic pathways; as learning and memory in mammals are among the most complex of biological processes, we can only expect their genetics to be complex.

We should not abandon genetic analysis because of its unwelcome message that learning and memory are more complicated than our heuristic theories allow. Nor should we hope that genetics or some future method of analysis will effect a miraculous simplification of these processes. Rather we should make use of, and integrate, data from all available methods. In the interplay between the production of mutants and their phenotypic analyses, our ability to generate mouse mutants has clearly outstripped our ability to analyse their phenotypes. We are especially in need of more refined methods for studying neural processes in mice.

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