

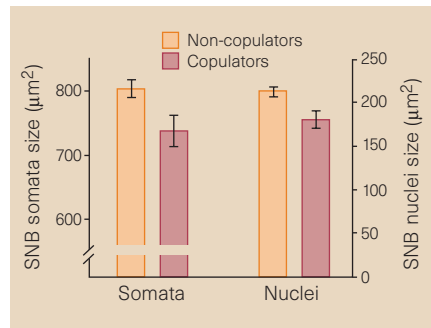
# Sex on the brain

Reports of morphological differences between the brains of humans with different sexual orientation<sup>1</sup> or gender identity<sup>2</sup> have furthered speculation that such behaviours may result from hormonal or genetic influences on the developing brain. However, the causal chain may be reversed; sexual behaviour in adulthood may have caused the morphological differences. I report how adult sexual experience alters the appearance of rat motor neurons as revealed by Nissl staining, the same technique used in post-mortem human studies.

Male Sprague–Dawley rats were castrated at 117–123 days of age and I implanted 5-mm-long testosterone-filled Silastic capsules subcutaneously, to produce a much lower than normal concentration of circulating androgen while sustaining continued male copulatory behaviour<sup>3</sup>. One week later I gave each rat as a cage-mate an ovariectomized female that had been subcutaneously implanted with either a 5-mm capsule containing 10% oestradiol to induce constant behavioural receptivity<sup>4</sup>, or an empty capsule.

Male rats provided with oestrogen-treated cage-mates began copulating shortly after the introduction of the receptive female and were therefore labelled ‘copulators’. The males caged with untreated (and therefore unreceptive) females were never observed to copulate and are designated ‘non-copulators’. Females were replaced from a reserve pool every 3 days (with confirmation that only the oestrogen-treated females were receptive) for 27 days.

I stained the spinal cords from the males



**Figure 1** Sexual experience alters neuronal morphology.

with thionin to reveal motor neurons in the spinal nucleus of the bulbocavernosus (SNB)<sup>5</sup>. These motor neurons and their striated target muscles are active during male copulatory behaviour<sup>6</sup>, and shrink after castration unless replacement testosterone is provided<sup>7</sup>. Changes in the size of SNB somata and nuclei are accompanied by changes in the number and size of synaptic inputs to the motor neurons<sup>8</sup>. An observer, blind to group membership, determined the number and cross-sectional area of SNB somata and nuclei<sup>7</sup>.

As expected, SNB somata and nuclei were smaller than in gonadally intact males<sup>7</sup> (Fig. 1), and there was no difference between the groups in the number of SNB motor neurons. The number of neurons, which is reported in only a few human studies<sup>9</sup>, appears stable. The ten copulator males had significantly smaller SNB somata ( $P < 0.04$ , two-tailed  $t$ -test) and nuclei ( $P < 0.02$ ) than the nine non-copulators.

The bulbocavernosus muscles innervated by the SNB were also lighter in copulators ( $711 \pm 37$  mg; mean  $\pm$  s.e.m.) than in non-copulators ( $868 \pm 50$ ;  $P < 0.02$ ). The animals received equivalent androgen exposure as shown by the lack of difference in either body mass ( $P > 0.20$ ) or the mass of the highly androgen-sensitive seminal vesicles ( $168.5 \pm 12.9$  mg, copulators;  $177.8 \pm 11.9$  mg, non-copulators).

Copulatory experience can therefore alter the size of neurons, as revealed by Nissl staining. Whether the sensory experience or motor activity of copulation induced these morphological changes, interpretations of correlations between human behaviour and neural morphology must acknowledge that the two are reciprocally related<sup>10,11</sup>. It is possible that differences in sexual behaviour cause, rather than are caused by, differences in brain structure.

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## Antibiotic resistance spread in food

Nutritive and therapeutic treatment of farm animals with antibiotics, amounting to half of the world’s antibiotic output, has selected for resistant bacteria that may contaminate the food produced. Antibiotic-resistant enterococci and staphylococci from animals are found in food when they survive the production processes, as in raw cured sausages and raw milk cheeses<sup>1</sup>. The broad host ranges of some plasmids and the action of transposons in many bacteria allow antibiotic-resistance genes to be communicated by conjugation between different species and genera<sup>2,3</sup>. A multi-antibiotic resistance plasmid from a lactococcus found in cheese provides a historical record of such events.

Lactococci function as starter cultures

for lactic fermentation in cheese and have conjugative ‘metabolic’ plasmids<sup>4</sup>. The streptomycin-, tetracycline- and chloramphenicol-resistant *Lactococcus lactis lactis* strain K214 was isolated by us in 1993 from a raw milk soft cheese ( $2 \times 10^8$  colony-forming units (c.f.u.) per g). A circular 29,871-base-pair plasmid (pK214) transferred all three resistances to *Enterococcus faecalis* strain JH2-2 by electroporation. We determined the complete nucleotide sequence of pK214 and identified homologous molecular structures in other organisms (Table 1).

A putative *Lactococcus* region of the plasmid contains genes for a resolvase, a replication-associated protein, a DNA nickase, insertion sequence IS904 and a new insertion-sequence element. A new membrane-spanning efflux protein confers increased macrolide resistance (erythromycin and others) on *Escherichia coli*, but not on strain K214 itself. A *repB* gene and tandem repeats

(iterons) identify pK214 as a theta-type replicating plasmid.

A mainly *Staphylococcus*-derived segment contains the information for the streptomycin-inactivating adenylase, the chloramphenicol-inactivating acetylase and replication protein RepD, known from staphylococcal plasmids. A mobilization gene is homologous to a gene from a *Lactobacillus plantarum* plasmid. The region is flanked on both sides by insertion-sequence elements from *Enterococcus faecium*.

The tetracycline-resistance gene (providing ribosome protection) is 99.8% homologous to *tet(S)* from *Listeria monocytogenes*. It is joined by sequences similar to a region of transposon Tn916 from *E. faecalis* which is involved in *tet(M)* expression. Another *E. faecium* insertion-sequence element complements the assembly.

Fifteen open reading frames (ORFs) including six insertion-sequence elements aid DNA rearrangement, regulation, mobi-

**Table 1 Homologies to sequences from the antibiotic-resistance plasmid pK214**

ORF	Position (base pair)	Highest homology	Sequence identity (%)	Organism	GenEMBL accession number
1	21–593	Resolvase	63.6	<i>Lactococcus lactis</i>	X99798
2	681–1448	Replication-associated protein SOJ	29.1	<i>Mycoplasma</i>	V34816
3	1451–1750	Unknown	-	-	-
4	1824–2189	Unknown	-	-	-
5	2,204–2,584	Unknown	-	-	-
6	2,610–3,554	Unknown	-	-	-
7	4,236–5,390	Replication protein RepB	82.3	<i>Lactococcus lactis</i>	L02920
8	5,868–6,185	Unknown	-	-	-
9	7,231–8,925	DNA nickase	24.4	<i>Streptococcus agalactiae</i>	L39769
10	9,206–10,358	IS904 (transposase)	99.8	<i>Lactococcus lactis</i>	D00696
11	10,534–11,790	Macrolide efflux protein	34.6	<i>Streptococcus pyogenes</i>	U70055
12	11,879–12,331	phnB protein	29.9	<i>Escherichia coli</i>	J05260
13	12,458–12,991	Protein p35	38	<i>Escherichia coli</i>	X13270
14	13,008–13,382	IS904 (truncated transposase)	100	<i>Lactococcus lactis</i>	D00696
15	13,439–14,899	IS150 (transposase)	40.8	<i>Escherichia coli</i>	X07037
16	15,678–15,127	Resolvase	78.8	<i>Staphylococcus aureus</i>	X16298
17	16,998–16,153	Regulation protein (Rob)	40	<i>Escherichia coli</i>	M97495
18	17,087–17,896	IS1216	98.2	<i>Enterococcus faecium</i>	L40841
19	18,908–18,060	Streptomycin adenylase	98	<i>Staphylococcus aureus</i>	X06627
20	19,755–18,916	Replication protein RepD	89	<i>Staphylococcus aureus</i>	X02166
21	21,663–20,492	Mobilization protein (Mob)	59	<i>Lactobacillus plantarum</i>	M33531
22	22,563–21,916	Chloramphenicol acetylase	99.9	<i>Staphylococcus aureus</i>	M90091
23	23,036–22,698	Replication initiator (truncated)	95.1	<i>Listeria monocytogenes</i>	X68412
24	23,133–23,941	IS1216	98.7	<i>Enterococcus faecium</i>	L40841
25	24,429–24,199	ORF 7 of transposon Tn916	56.2	<i>Enterococcus faecalis</i>	L15633
26	24,906–25,265	ORF 9 of transposon Tn916	53.6	<i>Enterococcus faecalis</i>	L15633
27	25,964–25,779	ORF 6 of transposon Tn916	80.3	<i>Enterococcus faecalis</i>	L15633
28	27,974–26,034	Tet(S)	99.8	<i>Listeria monocytogenes</i>	L09756
29	29,016–29,824	IS1216	99.6	<i>Enterococcus faecium</i>	L40841

Assignments of ORFs of the *Lactococcus lactis* antibiotic-resistance plasmid pK214 (GenEMBL accession no. X92946) to known genes and functions on the basis of DNA homology scores and derived amino-acid sequences. IS, insertion sequence. The complete nucleotide sequence of pK214 was obtained by the 'primer-walking' technique applied to overlapping fragments cloned in pUC18.

lization and replication. Seven ORFs have to do with antibiotic resistance and seven are unknown, ill-defined or truncated. No known plasmid-transfer genes are present. Whether conjugative mobilization of pK214 can be triggered by the other three plasmids of strain K214 (sizes roughly 10, 20 and 50 kilobase pairs) remains to be established.

In plasmid pK214, *Lactococcus* K214 has, with the help of insertion-sequence elements, collected genetic information from four other species to construct an antibiotic survival kit that also works in *E. faecalis*. pK214 is a live record of previous genetic exchange between pathogenic and non-pathogenic bacteria in food-associated environments. It is further demonstration of the presence of transmissible antibiotic-resistance genes in the human food chain. The resistant bacteria probably originated from antibiotic treatment of the cows. As lactococci may be found together with enterococci and staphylococci as part of the cows microflora, resistance transfer and pick-up may have occurred in the animals or during cheesemaking, where enterococci, listeria and staphylococci survive and eventually multiply.

When analysed in 1991 and 1995, this

cheese brand had also contained different enterococci resistant to tetracycline, chloramphenicol, gentamycin, penicillin, erythromycin, lincomycin and vancomycin (mechanism unknown but neither vanA nor vanB) at  $10^6$ – $10^7$  c.f.u.  $g^{-1}$  (ref. 1). This cheese was the source of a *Listeria monocytogenes* epidemic in 20 patients in 1995<sup>5</sup>.

To preserve the life-saving potential of antibiotics, the spread of resistance genes at all levels must be stopped<sup>6</sup>. Distribution routes like those between animals, food and consumers have to be interrupted. In this example, it could be achieved by using pasteurized milk for cheese making. The situation might also be helped by stopping the inappropriate use of the selective antibiotics in animal husbandry. Regarding the presence of transmissible antibiotic resistances in microorganisms from food, a rethinking of food safety issues has recently been proposed<sup>7</sup>.

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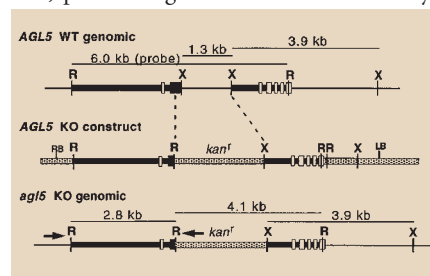
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## Targeted disruption in *Arabidopsis*

Homologous recombination has been used for two decades to target insertions into cloned genes in bacteria and yeast, and more recently has become a routine method of gene inactivation in mammals. *Arabidopsis* is one of several multicellular model organisms (along with *Drosophila*, *Caenorhabditis* and zebrafish) in which mechanisms controlling development have been studied. Previously, traditional genetic methods have been used, as targeted disruption by homologous recombination has not been successful in any of these organisms. We have now successfully disrupted the *AGL5* MADS-box gene in *Arabidopsis* by homologous recombination, providing a useful tool for future analyses.

Experiments in cultured *Arabidopsis* cells using a root transformation procedure have indicated that targeted disruption of cloned genes should be possible<sup>1</sup>. But regeneration was not possible from this cell line, prohibiting the isolation of homozy-



**Figure 1** Diagram of wild-type *AGL5* genomic region, knockout (KO) construct, and *agl5* mutant genomic region. Thick black lines indicate the genomic region included in the targeting construct. Dashed lines indicate the genomic region that was targeted for replacement by the kanamycin-resistance cassette (*kan<sup>r</sup>*). Also shown are the positions of oligonucleotide primers (arrows) used to identify the targeted insertion, and the probe used in DNA analyses. Exons are indicated by white boxes, the black box represents the MADS-box, and the stippled bar represents the pZM104A vector<sup>1</sup> including the *Agrobacterium* left (LB) and right (RB) T-DNA borders. R, EcoRI; X, XbaI.