

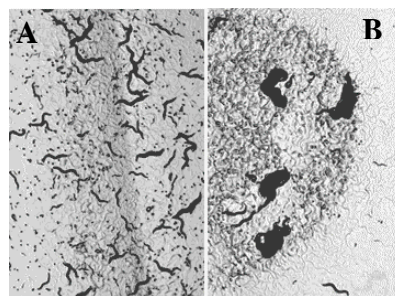
### A gene involved in nematode feeding behaviour

Recent studies on feeding behaviour in the soil nematode *Caenorhabditis elegans* provide a striking illustration of how genetic and environmental influences together mould complex behaviour.

*C. elegans* worms inhabit decaying organic matter in the soil and feed on different species of bacteria. Cassada first noted in 1987 that in the presence of food, wild strains of *C. elegans* exhibit one of two distinct feeding behavioural patterns, known as 'solitary' and 'social'. Since then, this has been confirmed in the case of every one of 17 natural isolates from all over the world (de Bono and Bargmann 1998). Solitary feeders forage alone on a bacterial lawn (figure 1A). The common laboratory strains N2 (originally isolated from England) and TR403 (from Wisconsin, USA) belong to this category. In contrast, social feeders tend to clump with each other while feeding (figure 1B). Some isolates from Hawaii, Australia, Germany and England show such social feeding. In one instance, social and solitary wild strains were isolated from the same sod of soil (de Bono and Bargmann 1998). It must be emphasized that the two classes of isolates differ in their feeding behaviour only under conditions of a plentiful supply of food bacteria, underlining the importance of the environment in determination of this behaviour. Also, the aggregation behaviour is enhanced by an increased density of worms (de Bono *et al* 2002).

A clue that the behavioural difference between solitary and social feeders might be correlated with variation at a single genetic locus came with the isolation of three mutants of the solitary strain N2 that showed social feeding (de Bono and Bargmann 1998). The three point mutations were all recessive to the wild-type and did not complement each other. This indicated that they shared the same locus. The mutations were mapped to a single gene on the X-chromosome, named *npr-1* (for *neuropeptide receptor* resemblance; see below). In other experiments, back-crossing was carried out for 10 generations between the naturally occurring social strain RC301 from Germany and N2. The progeny in every cross were selected for a marker close to the *npr-1* locus. The DNA of the quasi-inbred strains thus produced contained sequences from the social strain around the *npr-1* locus but had N2 sequences elsewhere. All the independently outcrossed strains exhibited strong social behaviour similar to the parental RC301 strain. Once again, it appeared that allelic variation in single region of the genome of the social strain, perhaps in just *npr-1*, was sufficient to change the behavioural phenotype (de Bono and Bargmann 1998).

NPR-1 is thought to encode a 7-transmembrane G-protein-coupled receptor, similar to members of the neuropeptide Y receptor family. With the exception of just one base position, the *npr-1* coding region of 1.4 kb was found to be identical in all 17 natural isolates tested. But the variation at that position



**Figure 1.** The solitary and social strains of *C. elegans* behave differently in the presence of bacteria. (A) Solitary worms disperse evenly across a bacterial lawn and browse alone. (B) Social animals aggregate together into clumps (de Bono and Bargmann 1998; courtesy, M de Bono).

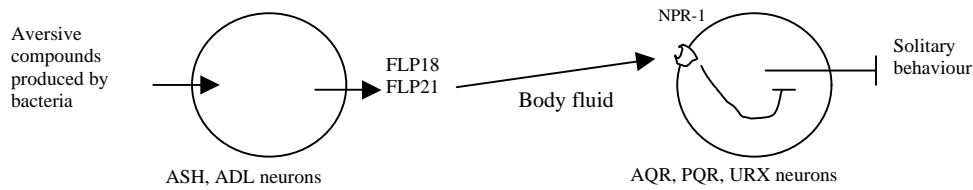
was critical. The twelve social feeding strains had a TTT codon [implying that phenylalanine (F) was amino-acid number 215 in the predicted NPR-1 sequence] in the same position at which the remaining five solitary strains had a GTT codon [implying that in them amino-acid number 215 was valine (V); de Bono and Bargmann 1998]. Thus despite their geographically distinct origins, the wild isolates displayed a perfect correlation between the 215th residue of NPR-1 and feeding behaviour, at least in the sample of worms tested. A comparison of the NPR-1 sequence with that of other G-protein-coupled receptors indicated that residue 215 was probably located in the third intracellular loop of the receptor. This loop is the site of protein-protein interaction and any change in sequence of this loop might alter G protein specificity and/or affinity and hence affect downstream signalling. The 'solitary' 215V allele is dominant over the 'social' 215F allele. Accordingly, expression of *npr-1* 215V from a transgene was sufficient to make a *npr-1* 215F worm behave like its solitary counterpart. But *npr-1* null mutants are hypersocial, implying that both the natural variants depress social feeding behaviour upon activation (de Bono and Bargmann 1998). *npr-1* 215V worms might be solitary because this isoform inhibits social feeding more potently than the 215F isoform.

Behavioural differences in populations can rarely be accounted for by variation at a single locus, and these studies would seem to belong to the rare class. However, it turns out that one should be cautious in coming to such a conclusion.

The simple nervous system of the *C. elegans* provides opportunities to investigate the role of nerve cells and their connections in fostering solitary or social feeding. An adult worm, about 1 mm long, contains 302 neurons. Each of these neurons can be uniquely identified by morphology and anatomical position. The first study investigated how and where NPR-1 acts. When a green fluorescent protein (GFP)-tagged version of the *npr-1* 215V allele was expressed under its native promoter in *npr-1* null (therefore hypersocial) worms, a number of neurons in the transgenic worms showed fluorescence – and, as mentioned earlier, the worms themselves displayed solitary feeding. By using different promoters to drive expression of the transgene in various subsets of neurons, it was inferred that expression in just three pairs of sensory neurons was sufficient to suppress social feeding (Coates and de Bono 2002). These three pairs of neurons, AQR, PQR and URX, are directly exposed to the pseudocoelomic body fluid. This opens the possibility that distant neurons in the body of the worm can regulate the activity of AQR, PQR and URX by secreting neuropeptides or neuromodulators in the body fluid – an observation that parallels the well-known fact that humoral signals regulate mammalian feeding.

The knowledge that both food and population density influence feeding behaviour helped in the design of another experimental strategy. A collection of genetically defined mutants that were impaired in the reception of various environmental stimuli like attractants, repellants and touch was screened for feeding behaviour. This led to the identification of four genes which, when mutated, also showed altered feeding behaviour (de Bono *et al* 2002). Two code for cation channel subunits in chemosensory neurons and are involved in avoidance behaviour to noxious stimuli such as benzaldehyde and 1-octanol. The other two genes encode factors required to localize chemosensory receptors to sensory cilia. Laser ablation of the nociceptive neurons ASH and ADL abolished social feeding behaviour in *npr-1* mutant worms. Streptomycin-killed *Escherichia coli* did not induce social feeding; but exposure to volatile substances from live *E. coli* cells did (de Bono *et al* 2002). Thus, bacteria seem to produce compounds detected by nociceptive neurons that stimulate social feeding and related behaviours. This led to the suggestion that aggregation during feeding might have evolved as a stress response to aversive stimuli produced by bacteria. It appears odd to think of food as a source of an aversive stimulus. On the other hand, *E. coli* and many soil bacteria that form part of the diet of *C. elegans* in nature can kill worms under certain conditions such as presence of certain virulence factors in bacteria, the age of the worm host and so on.

Recently, using a candidate-ligand approach, FMRFamide related neuropeptides (FaRPs) were identified as NPR-1 ligands (Rogers *et al* 2003). These neuropeptides (encoded by *flp18* and *flp21*) have the amino acid sequence of FMRF at their carboxy-terminus with an amide modification on the C-terminal Phe residue. Loss of function studies and overexpression of genes encoding these peptides showed that the peptides can influence feeding behaviour. Injection of chemically synthesized peptide in wild social worms strongly suppressed aggregation behaviour, confirming that NPR-1 ligands inhibit social feeding. The genetic and neurobiological data can be integrated to propose a hypothetical and preliminary circuit that underlies feeding behaviour (figure 2).



**Figure 2.** Schematic of a possible NPR-1 neural circuit: Bacteria emit an aversive odour that stimulates ASH and ADL neurons to secrete FLP-18, FLP-21 neuropeptides in the body fluid. AQR, PQR and URX neurons act to suppress solitary behaviour, promoting social feeding. These also express NPR-1, which binds FLP-18, FLP-21 in the body fluid. This activates NPR-1, which results in inhibition of neuronal activity of AQR, PQR and URX. NPR-1 215V isoform is more potent in inhibiting the neurons than 215F isoform. Thus neurons with 215V isoform fail to inhibit solitary feeding. Consequently, this isoform promotes solitary behaviour while the other isoform promotes social feeding (see text for more details and references).

What are the selective forces that affect feeding behaviour? Which (if any) of the two feeding patterns is more advantageous? The solitary and social feeders come from many locations, and in one case both were isolated from the same site. The two may represent alternative foraging strategies that are favoured under different – and as yet unknown – environmental conditions. In a given environment one strategy could be superior to the other. Alternatively, they may represent examples of two behavioural phenotypes that confer equal fitnesses when averaged over the spatial range of the populations or over the life cycle or both. In that case, they would constitute an interesting example of a genetic polymorphism underlying an evolutionary stable strategy (Gadgil *et al* 1982).

A comparison of *npr-1* sequences in different *Caenorhabditis* species shows that the 215F allele is widespread among closely related species (Rogers *et al* 2003). As mentioned earlier, the 215V allele is present only in a subset of *C. elegans* wild isolates. This points to the ‘social’ allele being more ancient than the ‘solitary’ one. Does this also mean that social behaviour is ancestral? It turns out that even when they carry the 215F allele, individuals from other *Caenorhabditis* species are solitary or weakly social feeders (M de Bono, personal communication). Therefore the correlation between the allelic state at the *npr-1* locus and feeding behaviour does not hold in other species. This means that other genomic loci (as already evident in the case of *C. elegans*) and the environment are important in shaping feeding behaviour.

Even though many questions remain, it is clear that *C. elegans* has much to offer in understanding how genes, neurons, circuits and the environment guide animal behaviour.

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