to mimic natural conditions. However, we have also demonstrated that, in the presence of food, genetic lifespan extension can be uncoupled from other life-history traits to the extent that fitness is not affected.

The observed fitness cost associated with field-like conditions provides an example of a single gene that acts in early life, ageing and Darwinian fitness, as predicted by the pleiotropy theory of ageing[2].

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Auditory perception

How cicadas interpret acoustic signals

The vertebrate ear can analyse the frequency components of sound with high resolution, recognizing complex acoustic signals even against a noisy background[1]. By contrast, insect ears can separate only broad-frequency bands, resulting in a categorical perception of sound[2]. We have discovered an insect, the cicada *Tettigetta josei*, that has a capacity for fine-frequency resolution, which could explain the evolution of frequency-modulated communication signals in cicadas.

Although known insects can discriminate only broad-frequency bands, their individual receptors can often respond selectively to frequencies within such a band and so might allow the central nervous system to perform fine-scale frequency analysis[3,4]. But this is not observed: receptor information is pooled either for distance estimation of an acoustic signal (exploiting the frequency-dependent attenuation of sound travelling in air[5]) or for sharpening the frequency band employed in acoustic communication by lateral inhibition[6].

Most hearing-insect groups have only a few auditory receptors in their tympanal organs (2–200; Table 1), which may constrain sound analysis in the frequency and time domains[1]. By contrast, cicadas have up to 2,100 auditory receptors in each ear, rivalling or even exceeding the number in most lower vertebrates (Table 1).

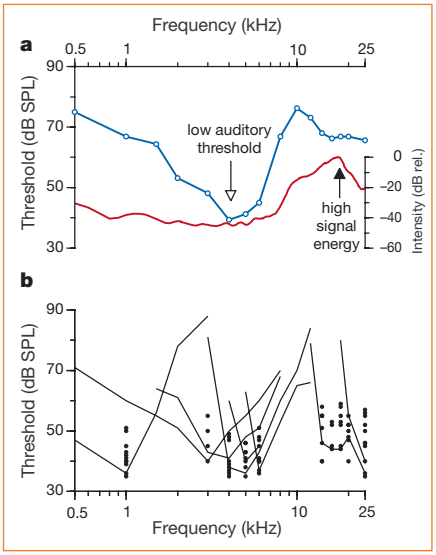

Figure 1 Song spectrum of the cicada *Tettigetta josei*, sensitivity of its auditory system, and sharp tuning of its interneurons to different frequencies. **a,** Threshold curve from extracellular auditory nerve recordings (circles; mean from $n = 9$) and spectrum of the song signal (average recorded from 8 males). The lowest threshold of this cicada's ear lies at 3–6 kHz (open arrow) and is not matched by the high-energy peak of the male signal at higher frequencies (filled arrow). **b,** Tuning curves from intracellular recordings made from 8 interneurons with different characteristic frequencies. Each point below 10 kHz represents the absolute threshold of an interneuron at its characteristic frequency. At frequencies above 10 kHz, some points stem from the same cell. Whereas recordings from the whole tympanal nerve reflect the threshold of the majority of auditory receptors (**a**), the differently tuned receptors remain undetected; however, these are revealed by sensitive interneurons at 1–1.5 kHz and 15–25 kHz (**b**). Full details of the methods are available as Supplementary Information.

Extracellular recordings of summed potentials of auditory receptors suggest that cicadas have a uniform tuning to a low-frequency band (3–6 kHz[7,8]), implying that for many of these insects the spectral energy of the relevant communication signals does not match the frequency range of best hearing (in 17 out of 22 species[7,8]). But differential tuning of these receptors in a cicada's ear might not only explain the apparent and — if true — maladaptive mismatch of the communication system, but also identify a function for the many receptors involved in frequency discrimination.

To test this idea of differential receptor tuning, we recorded intracellularly from auditory interneurons in the Portuguese cicada *T. josei*, which has a large number of receptors as well as a mismatch of frequency bands between the spectrum of the emitted signal and the apparent tuning of the auditory system (Fig. 1a). A set of interneurons tuned sharply to different frequencies shows that the thoracic nervous system is capable of frequency discrimination (Fig. 1b). At these frequencies, interneurons are extremely sensitive (thresholds of 30–50 dB sound pressure level) and many cells show 'roll-offs' of 30–50 dB per octave to one or both sides of their characteristic frequencies (a roll-off is a decrease in the sensitivity of auditory interneurons to adjacent frequencies, in octaves, to their characteristic frequency). These roll-offs convert to $Q_{10\ dB}$ values from 2.8 to 7.4 (Table 1, legend) which — for insects — are remarkably high and are similar to $Q_{10\ dB}$ values found for most lower vertebrates.

We conclude that these interneurons provide this cicada with a much lower threshold for intraspecific communication signals than would be predicted from recordings of the summed activity of auditory receptors, thereby solving the enigmatic frequency mismatch in the communication system of these insects (Fig. 1a). At

Table 1 Comparison of the tympanal ears of vertebrates and insects

	Number of auditory receptors	$Q_{10\ dB}$	*Frequency range (kHz)*
Vertebrates			
Fish	>5,000	0.35–1 (2)	0.05–1
Amphibians	14–1,500	0.3–3	0.1–4
Reptiles	50–2,000 (12,000)	1–7	0.01–5
Birds	5,800–9,600	2–12	1–10
Mammals	2,500–14,000	2–200	0.1–200
Insects			
Moth	2–4	<1	10–120
Mantid	20–35	<1	20–100
Bushcricket	20–60	1–2	0.1–80
Cricket	50–70	1–2	0.1–60
Grasshopper	30–80 (2,000)	~1	0.1–50
Fly	30–200	<1	1–40
Cicada	600–2,100	1–7	0.1–25

The number of auditory receptors in the ears of vertebrates (hair cells) and insects (scolopidial cells) is shown, together with the $Q_{10\ dB}$ values and frequency range within their auditory pathways. $Q_{10\ dB}$ values provide a measure of frequency tuning and are derived from a neuron's tuning curve at 10 dB above the threshold of the characteristic frequency (CF, the frequency at which a cell shows the lowest threshold); $Q_{10\ dB}$ is defined by CF divided by the bandwidth at 10 dB above the threshold of CF. The ranges given correspond to those commonly observed between species in a given group: numbers in parentheses are exceptions. References for the data shown are available as Supplementary Information.

frequencies of 1–6 kHz and 12–25 kHz, thresholds between cells with distinct characteristic frequencies differ by 20–30 dB at a given frequency (Fig. 1b), thus providing a unique facility (for insects) for frequency discrimination in the auditory pathway.

Given the uniform design of the auditory system in cicadas[7], it is possible that the frequency-modulated songs of many cicada species, particularly tropical ones[9], may result from sensory drive[10], because females are able to use frequency components of songs as criteria for species recognition and mate choice.

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Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

Biotechniques

Transfection of cells by immunoporation

Cell transfection is now a central technique in molecular biology and an essential prerequisite for gene therapy. Here we describe how beads coated with antibodies and bound to specific cell-surface transmembrane proteins can create holes in cells when the beads are removed, allowing transfection of the cells with DNA or other macromolecules. This unique targeted transfection of cells by immunoporation is very efficient and results in minimal cell death.

A variety of methods have been developed for the transfection of cells, including electroporation[1,2], lipofection[3,4], calcium phosphate coprecipitation[5,6] and DEAE dextran[7,8]. Of these methods, only electroporation offers the possibility of introducing DNA and other molecules such as proteins into viable cells. None of the current methods is able to target specific types of cells for transfection. In this new method

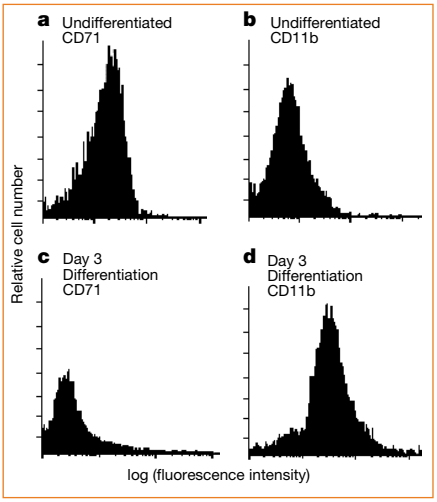

Figure 1 Analysis of the transfection of HL-60 cells with pEGFP-C1 by measurement of the expression of green fluorescent protein using flow cytometry. **a,** HL-60 cells transfected using DYNAFECT-CD71 beads; **b,** HL-60 cells transfected using DYNAFECT-CD11b beads; **c,** DMSO-induced HL-60 cells transfected using DYNAFECT-CD71 beads; **d,** DMSO-induced HL-60 cells transfected using DYNAFECT-CD11b beads.

of cell transfection, antibody-coated beads are bound to specific surface antigens and then the beads are sheared off from the cell by mixing: this causes the formation of transient holes in the cell membrane through which macromolecules can enter.

Granulocytic, differentiating human lymphoblastic HL-60 cells normally express CD71 on their surfaces. When induced to differentiate in the presence of dimethyl sulphoxide (DMSO), the cells cease to express CD71 and instead express CD11b. We have used this cell line as a model system to investigate the process of cell transfection mediated by immunoporation.

DYNAFECT beads coated with either anti-CD11b antibody (DYNAFECT-CD11b) or anti-CD71 antibody (DYNAFECT-CD71) were mixed on a rotating end-over-end mixer at 33 r.p.m. for 6 h at 22 ∞ C with either uninduced HL-60 cells or cells that had been induced with DMSO for 3 days. For mixing in a 2-ml microcentrifuge tube, 10^7 beads and 5×10^5 cells were suspended in 0.5 ml transfection medium (Dynal AS) containing 0.2 μg plasmid DNA vector pEGFP-C1 (4.7 kilobases) which codes for green fluorescent protein. After transfection, the beads were removed using a magnetic separator and the cells were transferred back into tissue-culture medium and cultured for a further 48 hours before analysis.

The extent of cell transfection was determined by flow cytometry. Figure 1 shows that the DYNAFECT-CD71 beads facilitate the transfection of DNA into normal HL-60 cells, but when the cells become differentiated and no longer express CD71, transfection no longer occurs with these beads. In contrast, mixing undifferentiated HL-60 cells with DYNAFECT-CD11b beads does not result in the transfection of the cells with DNA, but when the cells are differentiated and begin to express CD11b, those beads do bring about transfection.

Hence, immunoporation has the potential to target specific types of cell in a mixed population for transfection, depending on their immunological identity, and allow the targeted cells to take up a variety of different molecules. This also occurs in several mammalian cell lines with a range of different antibodies that target selected cell-surface antigens. In all cases, the level of transfection was 40–80%, depending on mixing conditions, and non-viable cells usually numbered less than 20%.

The high levels of selectivity and transfection, together with minimal cell death, that are achievable with immunoporation illustrate the enormous potential of this technique for use in a wide range of transfection studies. In particular, the ability to target specific subpopulations of cells will be extremely useful for many gene therapy applications.

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Erratum

Non-haemolytic β-amino-acid oligomers

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Nature **404,** 565 (2000)

Some symbols representing haemolytic activity were absent or incorrect in Fig. 1c. The correct figure is shown here. Crosses, β-17; circles, magainin derivative; squares, melittin.