demonstration that flow in a low-viscosity crustal channel that is coupled to surface denudation provides an internally consistent explanation not only for ductile extrusion of the GHS but for many other salient features of the Himalayan–Tibetan system. The critical factors are the presence of low-viscosity material in the middle to lower crust, a variation in crustal thickness between plateau and foreland, and surface denudation that is focused on the plateau flank. The range of model styles, and by implication the tectonics of natural orogens, is sensitive to variations in denudation rate and upper-crust strength. □

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Effect of acoustic clutter on prey detection by bats

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Bats that capture animal prey from substrates often emit characteristic echolocation calls that are short-duration, frequency-modulated (FM) and broadband[1]. Such calls seem to be suited to locating prey in uncluttered habitats, including flying prey, but may be less effective for finding prey among cluttered backgrounds because echoes reflecting from the substrate mask the acoustic signature of prey[2–4]. Perhaps these call designs serve primarily for spatial orientation[5–7]. Furthermore, it has been unclear whether the acoustic image conveyed by FM echoes enables fine texture discrimination[3,8,9], or whether gleaning bats that forage in echo-cluttering environments must locate prey by using other cues, such as prey-generated sounds[5–7,10–13]. Here we show that two species of insectivorous gleaning bats perform badly when compelled to detect silent and immobile prey in clutter, but are very efficient at capturing noisy prey items among highly cluttered backgrounds, and both dead or live prey in uncluttered habitats. These findings suggest that the short, broadband FM echolocation calls associated with gleaning bats are not adapted to detecting prey in clutter.

Two major and non exclusive[14–17] foraging tactics can be distinguished among insectivorous bats: aerial hawking (that is, the capture of airborne prey) and substrate gleaning. About one third of all microchiropteran bat species capture prey from substrates[15]. Unlike aerial-hawking bats that include longer-duration, and almost constant-frequency components in their echolocation calls, gleaning species emit calls that are often of low intensity and which often sweep from high to low frequencies (frequency-modulated (FM) calls) in a few milliseconds[1,4]. A major outstanding problem in echolocation biology is the extent to which these calls are used for distinguishing prey items from substrates, particularly when the substrate is complex and generates a lot of echo clutter[3] (that is, echoes from objects other than the target of interest). Some bat species emit calls at a high repetition rate ('feeding buzzes') to localize aerial prey, but switch off echolocation immediately before taking prey from surfaces: the bats may then listen instead for prey-generated sounds[7,11]. Most experiments on gleaning bats have investigated prey detection on simple surfaces, where background echoes may not mask prey echoes. In such situations, bats may still use echolocation to detect prey[18]. Bats may also use echolocation to detect prey positioned close to flat surfaces[19], and may even detect flying insects in grass by monitoring the insect's movement over successive echoes[20].

This study attempts to challenge the 'spectral image' paradigm[9] using a behavioural approach. We predicted that bats would use echolocation to locate aerial prey, and for trawling prey from smooth surfaces (mimicking water) with their feet. If FM echolocation is poorly adapted for prey detection in clutter, however, we predicted that bats would not use it for locating prey hidden in leaf litter because echoes from prey are masked by echoes from clutter. As a model species, we chose the sibling mouse-eared bats *Myotis myotis* and *Myotis blythii* because their ecology is especially well documented[15,21,22] and both emit FM calls with similar designs in every habitat configuration: while searching for prey, they emit calls that sweep from about 120–30 kHz in about 2 ms[23].

In the laboratory, wild-captured bats were provided with four artificial microhabitats, each mimicking foraging conditions faced by bats in nature[15,22]: (1) a complex, highly cluttered background provided by leaf litter (as in a deciduous forest); (2) a simpler, less cluttered background provided by an artificial lawn (as in a freshly mown meadow); (3) an acoustic mirror provided by a horizontal perspex plate (as with a still water surface); (4) no immediate background provided by prey items in the air (airborne prey). The ability of the bats to detect and capture live and dead prey items, respectively, was measured under these circumstances. The echolocation calls emitted by the bats were also recorded in each situation. Examples of video and sound sequences of foraging mouse-eared bats can be downloaded from our website (http://www.bio.bris.ac.uk/research/bats/myotis.htm).

A first experiment on detection cues attempted to establish how frequently moving, noisy prey were captured in comparison with dead prey. Mouse-eared bats foraging in clutter were much more proficient at capturing live, that is, moving and/or noisy prey, than dead prey (Fig. 1a). In a second experiment, we tested whether infrared illumination might influence the ability to detect prey. Success in capturing live prey was unimpaired in the absence of the infrared illumination necessary for video monitoring of bats' behaviour (Fig. 1b; distributions in Fig. 1a and b show no statistical difference, $\chi^2 = 2.54$, degrees of freedom, d.f. = 2, nonsignificant), demonstrating that mouse-eared bats do not rely on vision for prey detection. In a third experiment, we tested whether bats detected the rustling noise of live locusts crawling among dead leaves, or detected prey movements through echolocation. If passive listening is the dominant detection strategy when foraging in clutter, then the patterns of selection should be similar to those found in the previous experiments. If echolocation is used for prey capture, then there should be an equivalent number of prey captures for each situation. Bats easily found hidden rustling prey that were

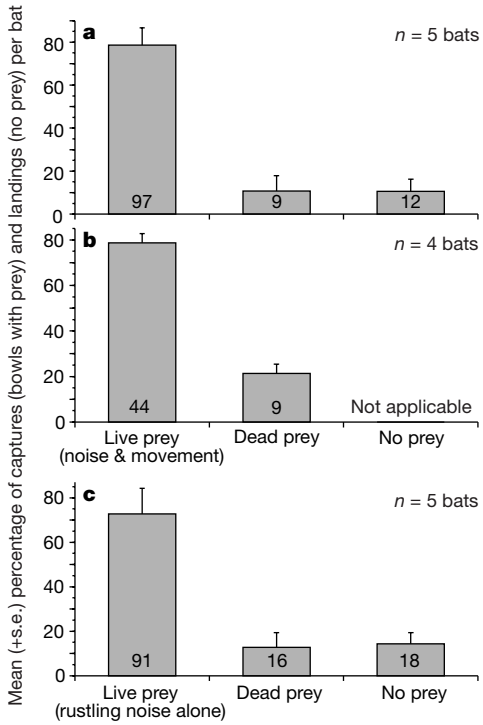

Figure 1 Experiments on foraging cues for experiments 1–3. See Methods. Data are presented as mean percentage of captures per bat ($n = 3$ *M. myotis* and 2 *M. blythii* bats; interspecific differences not significant); the standard error, s.e., indicates the inter-individual variance. The absolute number of captures (bowls with prey) or landings (no prey) is given at column foot (see Methods). **a**, Live prey generating both movement and noise, versus dead prey and no prey, under infrared illumination (two experiments per individual). **b**, Live versus dead prey in total darkness (no control in the absence of an infrared light; one experiment per individual). **c**, Live (noise-generating) prey hidden so as to render movement undetectable by echolocation, versus two controls (dead prey and no prey; each bat was submitted to this arrangement, over several trials if necessary, until 25 prey captures per individual were achieved) (infrared illumination).

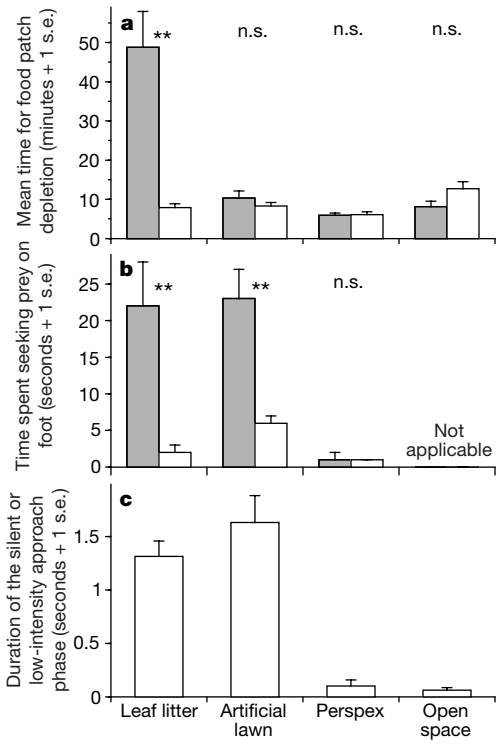

Figure 2 Experiments on foraging performance (**a**, **b**, under infrared illumination), and recordings of echolocation calls (**c**). Data were obtained from 4 *M. myotis* and 2 *M. blythii* bats. Data are presented as (**a**) average time (minutes) needed by a bat to deplete a patch containing five prey items, and (**b**) mean time (seconds) spent on foot searching for prey in the feeding arena (see Table 1 for further statistical details); s.e. represents the interindividual variance (each bat was offered three times dead and three times live locusts, and succession was randomized). Grey bars, dead prey; white bars, live prey. Probabilities are indicated by asterisks from *t*-tests performed with respect to prey status (dead versus live) for each experimental situation separately (double asterisk, $P < 0.01$; n.s., nonsignificant, **a** and **b**). In **a**, for dead prey, Tukey *post hoc* pairwise comparisons among situations yielded significantly greater values (that is, poorer performance) for leaf litter, compared with the other situations which did not differ from each other; in the case of live prey, performance was significantly poorer for open space than for leaf litter, lawn and perspex. In **b**, for dead prey, *post hoc* comparisons showed higher values (that is, poor performance) for both leaf litter and artificial lawn, compared with perspex. For live prey, artificial lawn gave a poorer performance than leaf litter and perspex. **c**, Duration (seconds) of the silent phase or low-intensity echolocation phase during approach of live prey in the same four experimental situations. *Post hoc* tests showed that the duration of the phase was significantly longer for leaf litter and artificial lawn (which did not differ from each other) compared with perspex and open space.

undetectable by echolocation (Fig. 1c): therefore, passive listening to prey-generated sounds, and not detection of prey movement by echolocation, is the basic tactic for foraging in clutter. This is because the bats' success in capturing live prey did not differ between the first and third experiment ($\chi^2 = 3.15$, d.f. = 2, non-significant).

When mouse-eared bats were required to detect silent, non-moving (dead) prey in maximum clutter (here represented by leaf litter) the average time needed to deplete the food patch was very much longer than for live prey (Fig. 2a). Moreover, when taking dead prey items in leaf litter, bats landed at random on the feeding arena and looked for prey by crawling around among dead leaves (Fig. 2b). Even a much simpler background such as an artificial lawn, with locusts protruding from the surface, yielded comparable results (Fig. 2b). Although time for patch depletion in these conditions was much faster than for leaf litter (Fig. 2a), presumably because the decision of a prospecting, flying bat to land was facilitated by the simple environmental structure, bats presented with dead locusts again landed on the feeding arena and sought prey by crawling (Fig. 2b). This suggests that the structure of the lawn was rendering the bats 'acoustically blind'. In contrast, mouse-eared bats performed much better when capturing both dead and moving locusts from the plexiglas surface, or in open space (Fig. 2a, b). Yet, the best performance overall was achieved either when bats gleaned moving, noisy prey in clutter, or when they caught prey of either status on a flat perspex surface, which mimics an acoustic mirror.

Bats produced feeding buzzes when localizing prey in air or on flat surfaces, but emitted no calls or only very faint calls at low repetition rate ('silent or low-intensity approach phase') for, on average, more than a second before detecting prey on complex surfaces (Fig. 2c). We suggest that the bats listen for prey-generated sounds during this phase. Accordingly, the low-intensity calls (optional) emitted during prey approach may detect the immediate surroundings so the bats avoid colliding with obstacles or the ground. Interestingly, a brief but intense buzz occurs immediately before landing, presumably allowing the bat to detect the ground (sound sequence on http://www.bio.bris.ac.uk/research/bats/myotis.htm): this provides further evidence that FM echolocation is not suited for prey detection in clutter (Table 1).

This study demonstrates first that mouse-eared bats rely on passive listening to prey-generated sounds, and not on echolocation, when foraging in echo-cluttering environments; second, that echolocation is of no use for detecting a non-moving, silent prey from cluttered backgrounds[20]. This is especially surprising as regards the artificial lawn which represents an apparently simple substrate. These findings contradict the view that the image conveyed by the echoes of FM calls can provide detailed information about the fine texture of objects, which would allow substrate-gleaning bats to distinguish prey from cluttered surroundings[9,24].

Table 1 Foraging performance and echolocation

Source of variation	Sum of squares	d.f.	Variance	*F* ratio	*P*
Time for food patch depletion					
Experimental situation	3633.7	3	1211.2	17.1	<0.001
Prey status (dead versus live)	1094.6	1	1094.6	15.4	<0.001
Situation × prey status	3995.4	3	1331.8	18.8	<0.001
Error	2835.0	40	70.9		
Time spent looking for prey on foot					
Experimental situation	0.572	3	0.191	17.721	<0.001
Prey status (dead versus live)	0.288	1	0.288	26.768	<0.001
Situation × prey status	0.286	3	0.095	8.880	<0.001
Error	0.430	40	0.011		
Duration of low-intensity phase					
Experimental situation	42.63	3	14.21	32.1	<0.001
Error	31.47	71	0.443		

Analysis of variance (ANOVA) carried out on foraging performance (mean time needed for depleting a food patch, Fig. 2a; time spent looking for prey on the surface of the arena, Fig. 2b), and duration of the phase of low-intensity echolocation calls during prey approach (Fig. 2c). As species was a nonsignificant factor in a preliminary analysis, the data from the two species were grouped together in all three cases. d.f., degrees of freedom; *F*, *F* statistics; *P*, probability.

Assuming that evolution has prompted mouse-eared bats to adopt the most efficient acoustic compromise[25,26], within the framework of their sensory system, there seem to be serious limitations to FM echolocation for prey detection in echo-cluttering habitats, apparently owing to the complexity of the overlapping reflected echoes. Under such circumstances, FM echolocation rendered the bats acoustically blind[13]. This suggests that FM echolocation is mainly adapted to orientation and capture of prey either in the open space or from simple backgrounds. □

Methods

Foraging cues

In experiment 1, we placed 15 plastic bowls, 30 cm in diameter and 10 cm deep, on the floor of the lightless flight room, spreading them regularly (3 × 5) over an area of about 6 m^2. The bottom of every bowl was covered with a 2–3-cm layer of leaf litter. One-third of the bowls contained five living—that is, moving and therefore sound-generating—locusts each; another five bowls contained five dead locusts each; and the remaining five bowls received no prey (control). The arrangement of the three types of prey availability on the floor of the flight room was randomized before each experiment. Individual bats were deprived of food during the preceding 24 h and released, one at a time, for 3 h into the experimental enclosure. Foraging behaviour was recorded using an infrared video and infrared lighting, which was the sole source of light. A landing into a bowl containing prey items always resulted in the capture of one prey item.

In experiment 2, we used the same design as above, but this time the bats had to seek food in total darkness (no infrared light). We counted the prey items left in the buckets at the end of the 3 h experiments. The proportions of dead versus live prey captured in the first and second experiments were compared.

Experiment 3 was similar to the previous ones, but the possibility of detecting prey movement by echolocation was removed: leaf litter was placed in all bowls, one third of which received two live, moving locusts each, one third two dead locusts each (first control), and one third no prey (second control), but this time all bowls were entirely covered with plastic mesh, on which was placed a second, thin layer of dead leaves and a dead locust. Only the latter was available to a foraging bat. A captured locust was replaced immediately, during the course of an experiment, whilst the bat was busy eating its prey from a perch.

Foraging performance and habitat clutter

We measured the foraging performance of bats, that is, the average time—the sum of the times elapsed from the beginning of the experiment until each prey capture, divided by the number of prey captured during the trial—needed to deplete a food patch, with respect both to the status of prey (dead versus live: that is, silent versus noisy prey) and the degree of environmental clutter (four artificial micro-habitats). Feeding arenas had similar dimensions (70 × 100 cm) in the first three experimental situations. Locusts were placed at random in the arena. Each bat was offered three times five dead locusts and three times five live locusts (six experiments per individual, randomized succession).

In experiment 4, prey were suspended by a thread 40 cm below a 70 × 100 cm wire structure hanging from the flight room ceiling; in order to imitate prey moving in the air (live prey), the frame was gently swung from outside the cage using a string. Experiments lasted for up to 100 min, but were interrupted if patch depletion occurred earlier. Analyses were performed using the average individual values obtained from three trials.

Acoustics

Bats were recorded capturing live prey. We used two bat detectors (S-25, Ultra Sound Advice) placed on each side of the feeding arena along the axis of the bats' flight path and recorded echolocation calls onto two channels of a high-speed tape recorder (Racal) at 76.2 cm s^{-1}. During the approach to prey, a light signal was generated towards the infrared video camera using a photographic flash beam covered with a blacklight filter. The flash (<2 ms) enabled synchronization of sound and flight sequences. Sound digitizing (sampling rate of 44.1 kHz; input speed divided by 16) and analysis of the duration of the low-intensity echolocation phase during prey approach (defined as the part of a sequence when call intensity suddenly drops; see Fig. 2c) were obtained by using Canary 1.2.4 (Cornell Bioacoustics Workstation).

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Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi

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Ancient asexuals directly contradict the evolutionary theories that explain why organisms should evolve a sexual life history[1,2]. The mutualistic, arbuscular mycorrhizal fungi are thought to have been asexual for approximately 400 million years[3,4]. In the absence of sex, highly divergent descendants of formerly allelic nucleotide sequences are thought to evolve in a genome[2]. In mycorrhizal fungi, where individual offspring receive hundreds of nuclei from the parent, it has been hypothesized that a population of genetically different nuclei should evolve within one individual[5,6]. Here we use DNA–DNA fluorescent *in situ* hybridization to show that genetically different nuclei co-exist in individual arbuscular mycorrhizal fungi. We also show that the population genetics techniques[4] used in other organisms are unsuitable for detecting recombination because the assumptions and underlying processes do not fit the fungal genomic structure shown here. Instead we used a phylogenetic approach to show that the within-individual genetic variation that occurs in arbuscular mycorrhizal fungi probably evolved through accumulation of mutations in an essentially clonal genome, with some infrequent recombination events. We conclude that mycorrhizal fungi have evolved to be multi-genomic.

Arbuscular mycorrhizal fungi (Class Zygomycetes; Order Glomales) are extremely successful fungi that form mutualistic symbioses with the roots of approximately 60% of all plant species[7]. They improve plant nutrition and promote plant diversity[8]. These fungi have been assumed to be asexual[7]. This is supported by measurements of the degree of linkage disequilibrium, which indicated that genetic variation among the spores of arbuscular mycorrhizal fungi deviates significantly from that expected from a recombinant population[4]. Genetic diversity in the ribosomal DNA occurs inside individual spores[9–12], even though it is thought that several copies of rDNA are kept the same by concerted evolution[13]. It has been hypothesized that by accumulation of mutations, in the absence of recombination, individual arbuscular mycorrhizal fungi have evolved to comprise genetically divergent nuclei, or that one individual contains several genomes[5]. Here we refer to an arbuscular mycorrhizal fungal spore as an individual.

We tested the hypothesis that individuals contain genetically different nuclei by performing specific fluorescent DNA–DNA *in situ* hybridization (FISH) on nuclei from spores of the arbuscular mycorrhizal fungus *Scutellospora castanea* (BEG 1). We used hybridization probes that specifically recognize two divergent sequences of the ITS2 region, known as T2 and T4, that were previously shown to co-occur within individual spores of this fungus[14]. Probes were only used for variant ITS2 sequences that had previously been shown to

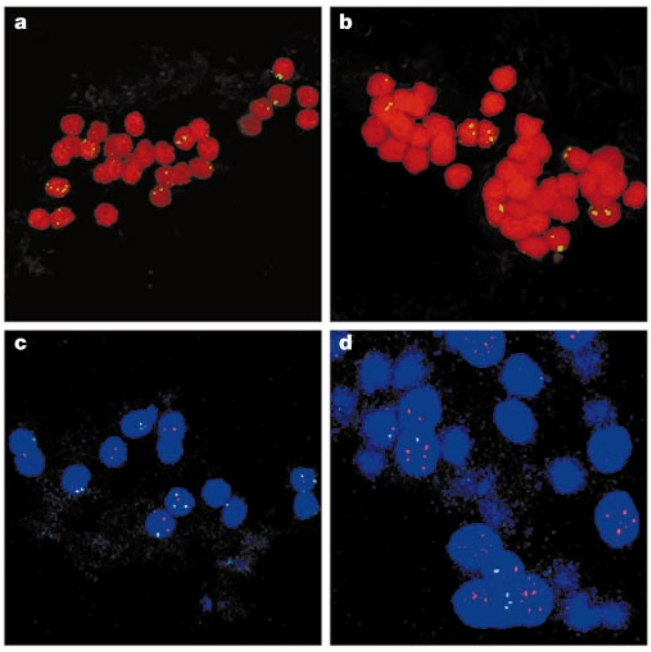

Figure 1 Nuclei of *Scutellospora castanea* taken with scanning laser confocal microscopy after single-target and double-target DNA–DNA FISH. **a**, Hybridization signals (green) of the probe T2-DIG to nuclei (red). **b**, Hybridization signals (green) of the probe T4-DIG to nuclei (red). **c**, Hybridization signals of the probes T2-DIG (light blue) and T4-biotin (red) to nuclei (purple). **d**, Hybridization signals of the probes T2-biotin (red) and T4-DIG (light blue) to nuclei (purple). The colours of images **c** and **d** have been adjusted to give better contrast between the colour of the two probes and the nuclei.