accompanying well data, in the Bengal basin has revealed that the Ganges–Brahmaputra delta started to grow rapidly ~40 Myr ago with an increase of sediment flux and development of significant prograding clastic depositional sequences. These sedimentation records are consistent with our view (based on the magmatism) that suggests an earlier rapid rise of eastern Tibet, from where thick piles of the lower sedimentary sequences in the Ganges–Brahmaputra delta and the Bengal fan could have been derived. A similar diachronous uplift history has been also proposed in the northern margin of the Tibetan plateau[27]. On the basis of radiometric dating results of granites and gneisses, Arnaud et al.[27] have argued that the basement unroofing and uplift in the eastern Kunlun Range occurred since ~35 Myr ago, and apparently preceded those in the western part of the Range.

Consequently, we suggest that the Tibetan plateau has undergone two main stages of rapid uplift caused by diachronous removal of thickened Asian lithosphere after the Indian indentation. Whereas the younger, and better-known, uplift began ~20 Myr ago in the western part of Tibet, the earlier event took place in the east, since ~40 Myr ago. Our observation can be reconciled with tectonic forcing models for the Cenozoic isotope evolution in the ocean[9] and global climate change[10]; these models put forward the effects of the rise and subsequent erosion and weathering of the Tibetan–Himalayan region. More specifically, it provides the first (to our knowledge) convincing time constraint that accommodates not only the rapid and steady increase in the seawater strontium isotope ratios beginning 40 Myr ago[9] but also the onset of global cooling from the early Eocene[10].

We further propose that the late Palaeogene tectonic processes in eastern Tibet played an important role in initiating the Ailao Shan-Red River shear zone, thus causing the continental extrusion[28] which eventually offset the Indochina block from the South China block for about 600 km (ref. 16). This may be due to the occurrence of a mechanically weakened lithosphere domain in the eastern Tibetan region as a result of the earlier lithospheric removal associated with extension and upwelling of hotter asthenosphere. The continental extrusion took place from about 27 to 17 Myr ago[16,29], nearly coincident with the motion of the Gangdese thrusting system in the southernmost Tibet[3,30]. Such a synchroneity relates to the long-standing debate on the relative importance of crustal thickening and lateral extrusion after the India–Asia collision. Future detailed investigations of this inaccessible mountainous region are needed for a more comprehensive understanding of the geological evolution of Tibet. In the absence of such data, any tectonic approaches using the one-stage uplift model for the entire Tibetan plateau may have to face the problem of oversimplification. ☐

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# Evolutionary transition from stretch to hearing organs in ancient grasshoppers

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**Ears of modern insects occur on a wide variety of body parts and are thought to have evolved from ubiquitous stretch or vibration receptors[1–4]. This relationship, based on comparative anatomy and similarities in the embryological development of ears in divergent taxa[5–7], has led to the widespread assumption of homology of these structures in insects, although this has not been tested rigorously. Here we report on the hearing organs of a relatively ancient[8], atympanate bladder grasshopper[9–11] (*Bullacris membracioides*), which is capable of signalling acoustically over ~2 km[12]. We show that, within single individuals of this species, serially repeated abdominal ears show functional continuity from simple to more complex forms. All 12 morphologically differentiated**

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organs respond to sound frequencies and intensities that are biologically significant, and mediate adaptive behavioural responses. By linking observations at the anatomical, physiological and behavioural level, our experiments provide evidence for the transition in function and selective advantage during the evolutionary development of this complex structure[13,14]. It is possible that ancestral insects with only simple pleural receptors had auditory capability covering distances substantially greater than contemporary insects with tympanate ears.

Although they lack differentiated tympana, bladder grasshoppers (Pneumoridae, Orthoptera) possess hearing organs homologous to those found in locusts. Histological staining shows that this organ contains nearly 2,000 sensory units (Fig. 1a). Multicellular scolopidia, each consisting of a bipolar sensory neuron and several accessory cells, are attached to the pleural cuticle of the first abdominal segment (A1) by means of two bundles of very long attachment cells (~1.4 mm in length compared with <100 μm in the tympanate hearing organs of modern grasshoppers). In addition, five pairs of smaller chordotonal organs form at the lateral body wall of abdominal segments A2–A6. Having only 11 scolopidia each, they attach by apical and basal cellular anchorages (Fig. 1b), with dendrites and attachment cells similar in length to those in A1.

We determined the neurophysiological function of both forms of abdominal chordotonal organ by recording extracellularly from nerve N1 of the respective ganglia. The male mating call is a resonant ($Q_{10\,dB} = 4.1$–$4.6$) call with a carrier frequency of ~1.7 kHz, comprising a resonant sixth syllable (98 dB sound pressure level (SPL) at 1 m) and five lower-intensity (70 dB SPL) introductory syllables[12]. We found that all abdominal chordotonal organs responded to acoustic stimulation within an intensity range that was biologically meaningful. Receptors in A1 have best frequencies of 4 kHz (Fig. 2), apparently mismatched to the 1.7 kHz carrier frequency of the male call[15–17]. Despite this anomaly and the absence of an overt tympanum, the sensitivity of the A1 hearing organ at its best frequency is one of the highest recorded so far for insects ($12.8 \pm 4.89$ dB SPL; $n = 5$) and, together with the intense calling songs, accounts for the extraordinarily large communication distance. In contrast, the tuning of receptors in segments A2–A6 matches the species-specific male signal (1.5–2 kHz) but they are

much less sensitive. Both individual tuning curves (Fig. 2) and mean threshold intensities (Fig. 3a) demonstrate increasing thresholds (58–77 dB SPL) from anterior to posterior segments. Nonetheless, the temporal song pattern of the male signal is detected in the spike discharge of all pleural chordotonal receptors and is faithfully represented when at suprathreshold intensity for a given segment (Fig. 3c; see A2 receptors).

It is the behaviour mediated by the serial chordotonal receptors, however, that establishes their role as functional hearing organs. First, we know from neurophysiological experiments with a biological microphone[18] (that is, a small, portable set-up for recording action-potential activity of auditory neurons directly in the field) that females have an average neural sensitivity of 32 dB SPL to the male call, and hear males calling at a distance of 1–2 km. However, in behavioural playback experiments, females show no auditory response until stimulation intensity exceeds 60 dB SPL (Fig. 3a). This in itself is not unusual as behavioural thresholds frequently exceed neurophysiological thresholds by such margins[19]. More unusual is a conspicuous sexual asymmetry in both the active space and the degree of stereotypy of mate location calls. Whereas females hear the male call with their most sensitive hearing organ in A1 over distances up to 1.9 km, males hear the soft female response over a maximum of only 50 m. Sound pressure levels of 60 dB, as perceived by females, imply that males are 50–100 m distant. This distance approximates the active space of the female call for the male, indicating that females risk auditory exposure to nocturnal predators only when a potential mate is clearly within her acoustic transmission range. There would have been strong selection for chordotonal receptor mechanisms that discriminate between 'advantageous' and 'disadvantageous' airborne sound signals and such mechanisms should evolve to maximize the expected utility of the receiver's response[20] in this antiphonal duetting system.

Second, playback experiments show that this adaptive behavioural response is graded. In contrast to the stereotyped male call, the female response varies from one to eight syllables. Behavioural tests showed this to be a function of male call intensity; beyond the response threshold females add approximately one additional syllable for each 3-dB increase in male call intensity (Fig. 3b). We examined the neurophysiological basis for this behaviour by testing the differential responses of pleural chordo-
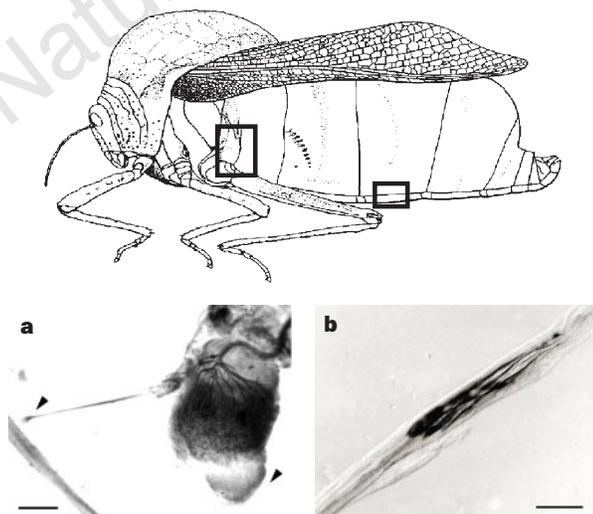


**Figure 1** Chordotonal organs in abdominal segments A1–A6 of adult bladder grasshoppers are differentiated morphologically, exhibiting two levels of complexity. **a**, Cobalt backfill of A1 reveals two cuticular connections (arrowed) with 32 scolopidia in the smaller branch and ~2,000 in the larger. Scale bar, 300 μm. **b**, Phase-contrast microscopy of a chordotonal organ with stained sensory cells attached to the pleura in A3. Scale bar, 50 μm.
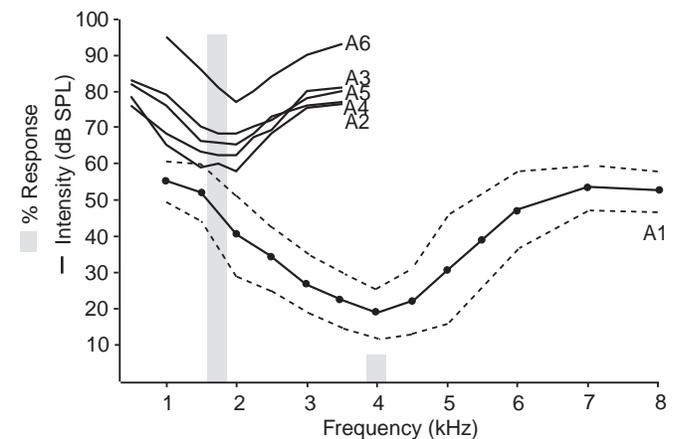


**Figure 2** Six pairs of abdominal chordotonal organs in adult pneumorids respond to sound with action-potential discharges at frequencies and intensities that are biologically significant. Neurophysiological tuning curve of A1 is for males and females combined (dotted lines indicate standard deviation limits of the mean tuning curve; $n = 11$). The curves for A2–A6 are from a single individual and thus do not reflect the means presented in Fig. 3a. Shaded bars demonstrate reliable behavioural responses of adult females to pure tones of 1.7 kHz and the paucity of response at 4 kHz. Note that an SPL of 75 dB at 4 kHz may be suprathreshold for some pleural organs.

tonal organs in females using playbacks of the male call, which simulated sender–receiver distances from 5 m to ~100 m. As a consequence of the different thresholds of the organs in A2–A6, the action-potential discharge representing the temporal pattern of the male call varies between the serially repeated segments at a given distance (Fig. 3c). How far away the male is from the female is thus, at least partly, encoded in the output of serially repeated sensory organs and the female's response would be subject to strong directional selection.

We have shown that the serial receptors in A1–A6 of *B. membracioides* fulfil all three criteria for functional ears: morphological, neural and behavioural[14]. That they are all true ears is further supported by results from ablation experiments in which we removed the A1 organs bilaterally from previously responsive females. Subsequent neurophysiological experiments confirmed the total absence of neural activity in the A1 afferent nerves of these individuals, but bilaterally operated females continued to respond to male calls at distances from 8–16 m in playback experiments. In addition, behavioural playback experiments to intact animals provide more direct support for the role of A2–A6 in mediating the adaptive female response. By using presentations of pure tones of 1.7 kHz and 4 kHz, we found that females responded almost invariably and exclusively to the lower frequency (96% versus 8%, $n = 76$ presentations; Fig. 2). This was despite a stimulus intensity that was ~40 dB above the hearing threshold at 4 kHz for the organ in A1, and <15 dB above that at 1.7 kHz for the organs in A2–A5. The few responses observed at 4 kHz most likely result from suprathreshold activation of pleural organ receptors at this frequency, rather than of A1 receptors. Ancestral insects with only simple pleural receptors may likewise have had auditory capability over distances higher than those for effective substrate-borne vibration, and substantially greater than many contemporary insects with tympanate ears.

We believe that these results illustrate the transition in function and selective advantage occurring during the evolution of complex suites of interdependent characters. Several lines of evidence indicate that chordotonal hearing organs represent a transitional form rather than a specialization unique to *B. membracioides*, although we do not suggest that the organs in *Bullacris* are themselves intermediate. Neurophysiological experiments on this species show that both chordotonal and auditory organs are sensitive to respiratory movements, highlighting their common origin from receptor cells involved in the perception of abdominal ventilatory movements[21,22]. Despite continuing questions concerning the precise phylogenetic placement of the Pneumoridae within the Pneumoroidea, all members of the superfamily lack tympana and possess a stridulatory apparatus, but only bladder grasshoppers have evolved an inflated abdomen and conspicuous calling. Abdominal inflation *per se* is not essential for the operation of chordotonal hearing organs, as demonstrated by females and alternate males[11] that lack this feature but share the same two forms of functional ears. Comparative data from other Pneumoroidea are necessary to assess the significance of conspicuous signalling for the evolution of chordotonal organs with auditory function, but clearly the evolution of specialized tympana and auditory organs need not be closely correlated. Molecular phylogenetic analyses suggest that the Pneumoridae probably arose in the Jurassic, before the tympanate Pyrgomorphidae, and from ancestors in which external tympana also appear to be absent[8]. We have examined the more basal atympanate Proscopiidae and although we should be cautious in declaring the absence of a structure, to date we have found no evidence of either ear form. It is likely that the pneumorid lineage has never contained species with classical ears, and that the extant species have retained an ancient form of hearing that represents a transitional stage in the evolution of insect ears.

Developmental and comparative studies of eared and earless insects have demonstrated modularity in structure and serial homology of auditory and mechanosensory receptors, suggesting that tympanic receptors might be a special part of the general chordotonal system common to all insect groups. To this modularity in structure we now add that of function. We propose that the functional continuity between classical auditory and pleural chordotonal organs in *B. membracioides* typifies the evolutionary trajectory of transitional forms from ancestral proprioreceptors to long-distance auditory receptors, which has occurred independently in divergent insect taxa. □
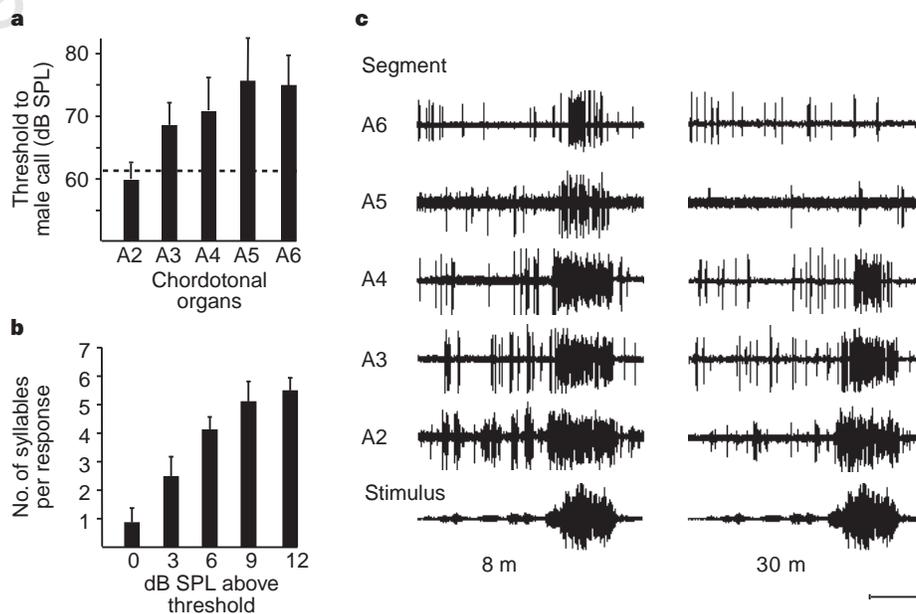


**Figure 3** Chordotonal organs mediate adaptive behavioural responses to acoustic signals. **a**, Mean neurophysiological thresholds of organs A2–A6 of females ($n = 10$) to the male call. Note the close correspondence between the A2 threshold and the behavioural response threshold (dashed line). **b**, Behavioural response of females to playback of male calls that are between 0 and 12 dB above response threshold. Females add approximately one syllable to their auditory response for each 3-dB increase in SPL. **c**, Differential neurophysiological responses of pleural chordotonal organs in females to playbacks of a male call simulating sender–receiver distances of 8 and 30 m in the field. Scale bar, 500 ms.

# letters to nature

## Methods

**Neurophysiology.** Experimental animals were collected at Inchanga (Kwa-Zulu/Natal, South Africa) and all field experiments conducted in February 1994–96 and March 1998. Numbers of sensory cells were determined either by backfilling the axons with cobalt lysine and intensifying the staining with silver according to standard methods[23] (for A2–A6), or by counting the number of attachment cells in 5-μm cross-sections of the chordotonal organ stained with methylene blue (for A1). Extracellular recordings of the afferent activity of pleural chordotonal organ receptors were obtained with silver-wire hook electrodes attached to nerve N1 of the respective neuromeres of the metathoracic ganglion or abdominal ganglia. Tuning curves for A2–A6 receptors were obtained by stimulating the preparation with digitized, pure-tone sounds pulses (sampling rate 44 kHz; 50 ms duration) of variable frequency (0.5–8 kHz) and intensity. Tuning curves of A1 receptors were established in a sound-damped chamber in Graz as previously described[24]. To determine how well the time pattern of the male signal is represented in the spike discharge of female chordotonal organs, we stimulated receptors in A2–A6 with digitized models of the male call (sampling rate 44 kHz) at SPLs ranging from 55 to 84 dB, thus simulating different sender–receiver distances.

**Behaviour.** Playback experiments were conducted as described[12]. Chordotonal organ ablation was done on anaesthetized females by opening the body wall at the attachment site of the hearing organ in A1, removing the organ and sealing the opening with histoacryl. Control animals were similarly sham-treated. Bilaterally operated females were tested the following day using playbacks of the male call at various intensities. Threshold was defined as the SPL of the male call that elicited at least one female chirp in response to the male call in four out of five presentations. In all ablation experiments we subsequently confirmed the absence of neural activity in the afferent nerves of the receptors in A1 with neurophysiological methods. Behavioural tuning experiments were performed in a sound-damped room using digitized, pure-tone sound pulses (sampling rate 22 kHz; 880 ms duration) of 1.7 and 4 kHz, randomly presented at 75 dB SPL at the position of the female and intervals of 40 s.

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# A proposed path by which genes common to mammalian X and Y chromosomes evolve to become X inactivated

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**Mammalian X and Y chromosomes evolved from an autosomal pair; the X retained and the Y gradually lost most ancestral genes[1,2]. In females, one X chromosome is silenced by X inactivation, a process that is often assumed to have evolved on a broadly regional or chromosomal basis[3]. Here we propose that genes or clusters common to both the X and Y chromosomes (X–Y genes) evolved independently along a multistep path, eventually acquiring dosage compensation on the X chromosome. Three genes studied here, and other extant genes, appear to be intermediates. ZFX, RPS4X and SMCX were monitored for X inactivation in diverse species by assaying CpG-island methylation, which mirrors X inactivation in many eutherians. ZFX evidently escaped X inactivation in proto-eutherians, which also possessed a very similar Y-linked gene; both characteristics were retained in most extant orders, but not in myomorph rodents. For RPS4X, escape from X inactivation seems unique to primates. SMCX escapes inactivation in primates and myomorphs but not in several other lineages. Thus, X inactivation can evolve independently for each of these genes. We propose that it is an adaptation to the decay of a homologous, Y-linked gene.**

By studying differences and similarities among homologous genes in extant species, one can draw inferences about ancestral genes and map points of evolutionary divergence. In this manner, one can explore the coevolution of the X and Y chromosomes and the evolution of epigenetic phenomena such as X inactivation. We first examined the inactivation status of individual X-linked genes in a wide range of mammalian species. Specialized reagents that are analogous to those used to assess X inactivation in humans and mice are unavailable for other species[4]. However, methylation of CpG islands, which exist at the 5′ ends of many genes[5], proved to be a widely applicable alternative. For human and murine X-linked genes, a perfect correlation has been observed between 5′ CpG-island methylation and X-inactivation status: transcriptionally silent, X-inactivated alleles are methylated, whereas active alleles are unmethylated[6]. To determine whether this correlation extends to a broad range of eutherians, we examined CpG-island methylation at *ALD*, an X-linked gene known to be X-inactivated in humans[7,8]. Eighteen species representing nine eutherian orders were tested. In all 18 species, methylation was observed in females, where *ALD* is presumably silenced on the inactive X chromosome; no methylation was observed in males, where the single X chromosome is active (Figs 1 and 2). This suggests that CpG-island methylation accompanies X inactivation in a wide range of eutherians.

We then explored the status, in diverse mammals, of three X-