

wedge. We propose that the strain intensification of each forethrust is a product of the build-up of the surface slope behind the forethrust and the tapered geometry of the wedge. □

Methods

We used the commercial finite-element software package, ABAQUS, to calculate the plastic strains and displacements in our models²⁸. The yield function, *F*, is defined as:

$$F = aq^b - p - p_t \tag{1}$$

where *p* is the mean stress, *q*, the equivalent Mises stress, is defined as:

$$q = \sqrt{\frac{3}{2} \sigma'_{ij} \sigma'_{ij}} = \sqrt{3J_2} \tag{2}$$

*J*₂ is the second variant of deviatoric stress, where $\sigma'_{ij} = \sigma_{ij} - p\delta_{ij}$ is the deviatoric stress tensor. *p*_{*t*} represents the mean pressure tensile strength of the material. *a* and *b* in equation (1) are material parameters, independent of plastic deformation, that define the concavity of the yield surface in stress space. The three material parameters defined above (*a*, *b*, and *p*_{*t*}) are determined by a least-squares fit to triaxial test data. Data for our models were limited to those studies done on sedimentary rock under at least three different confining pressures no larger than about 250 MPa, or approximately 10 km depth. We used yield stress versus confining pressure values for a sandstone²⁹ that were about average for all the sedimentary rocks for which data exist. The material parameters in equation (1) are *a* ≈ *b* ≈ 1 and *p*_{*t*} ≈ 0, which is identical for most of the yield stress data used in our models. The equivalent plastic strain measure^{28,30} is defined as:

$$\epsilon^p = \int \sqrt{\frac{\sigma_{ij} d\epsilon_{ij}^p}{p}} \tag{3}$$

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New light shed on the oldest insect

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Insects are the most diverse lineage of all life in numbers of species, and ecologically they dominate terrestrial ecosystems. However, how and when this immense radiation of animals originated is unclear. Only a few fossils provide insight into the earliest stages of insect evolution, and among them are specimens in chert from Rhynie, Scotland’s Old Red Sandstone (Pragian; about 396–407 million years ago¹), which is only slightly younger than formations harbouring the earliest terrestrial faunas. The most well-known animal from Rhynie is the springtail *Rhyniella praecursor* (Entognatha; Collembola), long considered to be the oldest hexapod^{2,3}. For true insects (Ectognatha), the oldest records are two apparent wingless insects from later in the Devonian period of North America^{4,5}. Here we show, however, that a fragmentary fossil from Rhynie, *Rhyniognatha hirsti*, is not only the earliest true insect but may be relatively derived within basal Ectognatha. In fact, *Rhyniognatha* has derived characters shared with winged insects, suggesting that the origin of wings may have been earlier than previously believed. Regardless, *Rhyniognatha* indicates that insects originated in the Silurian period and were members of some of the earliest terrestrial faunas.

Traditional interpretation of palaeontological evidence indicates that insects had their beginnings in a group of small, scurrying hexapods in the earliest Devonian period. The most basal insects are bristletails and silverfish, wingless scavengers with ectognathous mandibles and elongate, well-developed ovipositors^{6,7}. Until this study, true insects had not been known conclusively before the Emsian and consisted of only two records from the Devonian period. Cuticular fragments attributable to a wingless insect (either Archaeognatha or Zygentoma) were discovered in compressed shales of the Late Devonian of Gilboa, New York (approximately 379 million years (Myr) ago)⁵. Unfortunately, these isolated sclerites cannot be assigned definitively to any particular order of basal insects, greatly limiting their utility for understanding early insect diversification. Slightly older are the compressed shales of Gaspé Bay in Quebec, Canada (about 390 Myr), from which was recovered a bristletail (Archaeognatha)⁴. Curiously, the Gaspé fossil is not compressed unlike most digested fragments from such shales, leading some authors to contend that it is a recent contaminant⁸. A critical re-study of the Gaspé material is necessary.

letters to nature

At the time that *Rhyniella* was discovered and recognized as a springtail, a pair of well-preserved mandibles was identified among other sclerotized debris in a separate piece of chert. The mandibles were later studied by Tillyard who described them as *Rhyniognatha hirsti*⁹. Tillyard noted that *Rhyniognatha* was insect-like but he could not determine its placement within Hexapoda. Later authors also indicated the “suggestive” appearance of the mandibles but no detailed re-description or study was undertaken and conclusive placement of the fossil was left open; indeed, no subsequent authors re-examined the fossil, simply reiterating Tillyard’s assertion⁶. Thus, the Gaspé bristletail was reported as the earliest insect⁴ and *Rhyniognatha* was relegated to fragments of indeterminate origin¹⁰. Indeed, *Rhyniognatha* was even excluded from the most comprehensive account of hexapod fossils¹¹. Again without ever observing the original material, a recent account of the history of insects dismissed *Rhyniognatha* as either being a contaminant or representing some other arthropod¹². The authenticity of the material from Rhynie has already been elaborated elsewhere¹³ and, as we discuss below, the mandibles cannot be assigned to non-hexapod, mandibulate lineages.

Our study of the unique *Rhyniognatha* specimen in the Natural History Museum, London (NHM-Palaeontology, In. 38234), using

compound microscopy has revealed an anterior mandibular articulation, forming a socket (that is, an anterior acetabulum) on the inner angle of the mandibles, demonstrating that they are dicondylic (that is, with two points of articulation and restricted to a single plane of motion) (Fig. 1). *Rhyniognatha* is not transversely oriented or multiarticulated as in Crustacea, but among myriapods might most easily be confused with the millipede gnathal lobe. The millipede mandible is a robust, multiarticulated structure with a broad, sclerotized base to which the toothed gnathal lobe articulates. The gnathal lobe has a large, basal rasping surface, which is not present in *Rhyniognatha*. Furthermore, no evidence of additional, articulated mandibular sclerites exists in *Rhyniognatha* (Fig. 1). Such a mandibular construction is known only within the insects and corresponds to a monophyletic lineage comprising the silverfish (*Zygentoma*) and all winged orders (that is, Pterygota)^{6,7}. Thus, *Rhyniognatha* is definitively an insect. The stoutly sclerotized mandibles are surrounded by a suite of other sclerotized debris. Various tissues appear to represent fragments of the head capsule, whereas others ventral to and extending beyond the mandibular apices appear to represent maxillary fragments (perhaps portions of laciniae; Fig. 1). Extending from the base of the mandibles are large fan-like structures, which may have been portions of apodemes or

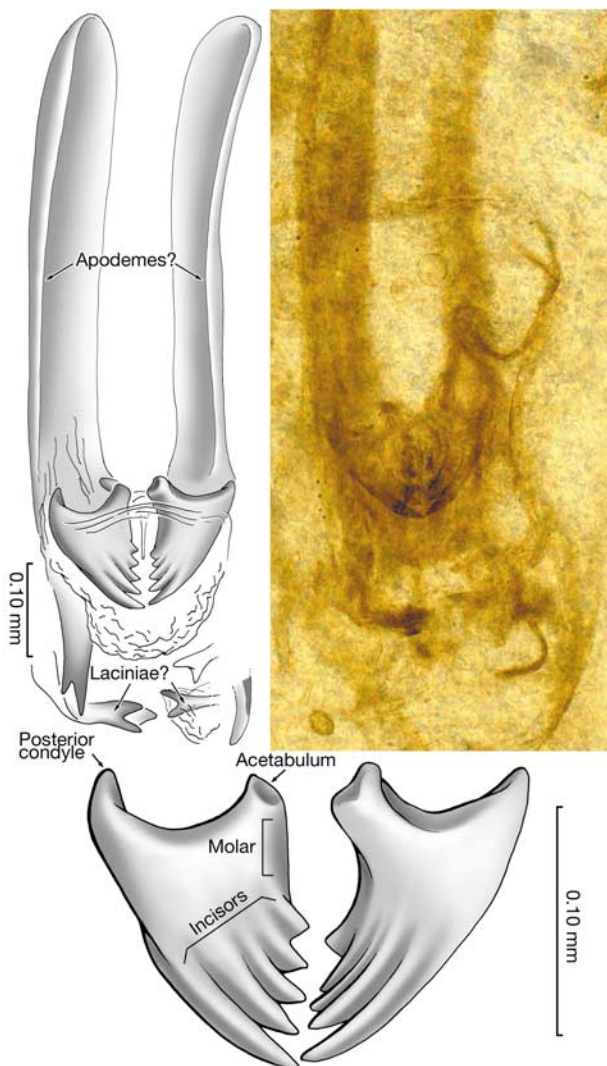


Figure 1 Remains of the oldest fossil insect. Holotype of *Rhyniognatha hirsti*, preserved in Early Devonian (Pragian) chert of Rhynie, Scotland. Photomicrograph of holotype and rendering of preserved remains, with enlarged detail of mandibles.

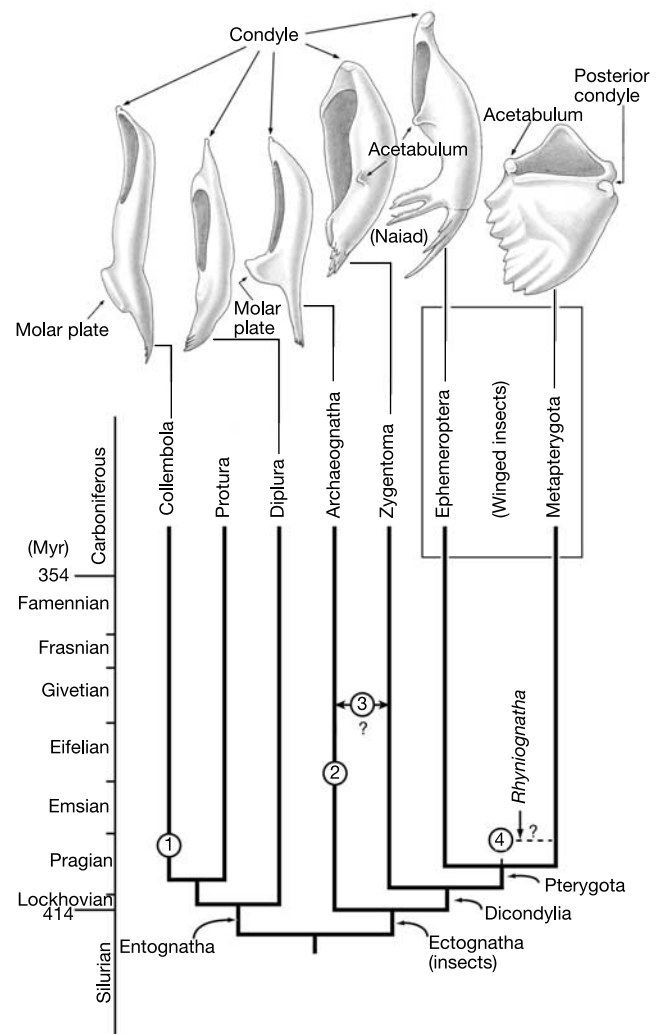


Figure 2 Phylogeny of basal hexapod orders¹⁶. Mandibular structure across the orders and putative position of *R. hirsti* (4) and other Devonian hexapods indicated (1, *Rhyniella praecursor* from Rhynie, Scotland; 2, putative bristletail from Gaspé, Quebec; 3, bristletail or silverfish remains from Gilboa, New York). Metapterygota (the clade consisting of Odonoptera and Neoptera) is represented by an orthopteran mandible.

perhaps even components of a tentorium. A more elaborate treatment of the surrounding sclerites will be presented elsewhere. Most remarkable is that the mandibles are short and triangular, rather than the elongate mandibles of Entognatha, Archaeognatha and Zygentoma (Fig. 2). In fact, this short and triangular morphology is typical of some Pterygota, suggesting that *Rhyniognatha* may have been a winged insect, although the definitive apomorphy, wings, are missing.

The most plesiomorphic lineage of winged insects are the mayflies (Ephemeroptera), which retain a subimaginal moult, very long cerci, and a median caudal filament, among other traits. Adult mayflies have vestigial mouthparts; however, naiads have generally primitive mandibles: these are elongate, with the posterior condyle (homologous with the articulation in Archaeognatha) set posteriorly along an extension of the mandible. The anterior articulation of mayflies is, by contrast to other dicondylid, winged insects, relatively weakly developed and as a result functions more similarly to a hinged joint^{14,15}. The complete fixation of the anterior articulation by a ridged ball-socket joint is a defining feature of the Metapterygota¹⁴⁻¹⁶ (that is, Odonoptera and Neoptera). The mandibles of *Rhyniognatha* also surprisingly resemble metapterygotes by the close position of the posterior condyle to the anterior acetabulum, the latter structure being well developed (Fig. 2) and distinctly unlike the weakened hinge of mayflies. *Rhyniognatha* consists of dicondylid mandibles that are not elongate, but are short and triangular as in basal metapterygote orders, and differs from basal insects (Ectognatha) as follows: from Entognatha and Archaeognatha it differs by the dicondylid mandibular structure; from Zygentoma it differs by the shortened and triangular metapterygotean construction of the mandible, discontinuous molar and incisor areas, and absence of a molar brush (also absent in Lepidotrichidae); it further differs from Ephemeroptera by the functional ball-and-socket joint of the anterior articulation (Fig. 1). The incisor and molar areas of *Rhyniognatha* are well differentiated, the incisor bearing stout, sharp teeth and the molar formed by a broad toothless region (Fig. 1). An anterior acetabulum (forming the anterior articulation) does not occur in any basal hexapod order except silverfish (Zygentoma) and the winged insects, hence Hennig's recognition of the latter two as a group called Dicondylia⁶. The diversity of insect lifestyles and diets is reflected in the myriad functional types of insect mouthparts¹⁷. Given the generalized structure of *Rhyniognatha*'s mandibles and presumed other mouthpart fragments this was clearly a chewing insect, but whether its diet was spores/pollen, leaf/stem tissue or small animals is impossible to say.

The metapterygotean structure of *Rhyniognatha*'s mandibles has profound implications regarding insect evolution. Current fossil evidence indicates that insect wings originated in the Early Carboniferous period, some 90, 170 and 270 Myr before pterosaurs, birds and bats, respectively, and these structures are believed to have led largely to the spectacular diversification of insects. Fossil insects are completely absent from the Late Devonian and Early Carboniferous, and a significant diversity of palaeopterous and neopterous species appeared suddenly in the earliest Late Carboniferous^{18,19}. Besides the derived structure of *Rhyniognatha*'s mandibles, further support for winged insects 80 Myr earlier than previously known pterygotes is provided by a recent DNA study²⁰. That study estimated that insects originated 434 Myr ago (in the Early Silurian) and pterygotes originated 387 Myr ago (in the mid-Devonian) (note, however, that only neopteran species were used to represent the entire pterygote lineage, and the dating actually applies to neoptery rather than wings, which must be older).

A Devonian origin of winged insects is highly relevant to several current hypotheses on the origin of wings. One hypothesis is that metabolically expensive insect flight evolved in hyperoxic atmospheres similar to what occurred in the Carboniferous and Permian²¹, but the oxygen content of Devonian atmospheres was far

less, approximately 15%²². Also, the paranotal theory hypothesizes that insect wings evolved from lateral extensions of the thorax, called paranotal lobes (but see also the exite theory²³), originally used for controlled gliding similar to what modern silverfish are capable of²⁴. Under this hypothesis, paranotal lobes presumably evolved later into broader, hinged structures capable of powered flight, analogous to the transitional forms seen with *Archaeopteryx* and modern birds²⁵ and with 'flying lemurs' (Dermoptera) and bats²⁶. Gliding requires a perch, and there is abundant evidence that various Palaeozoic insects grazed on spores from sporangia²⁷, which in most Devonian plants were produced at the branch tips^{28,29}. In fact, all of these spore feeders had generalized, chewing mandibles, not unlike those of *Rhyniognatha*. If winged insects appeared in the Early Carboniferous this would be approximately 30 Myr after arborescent plants evolved^{28,29}, but *Rhyniognatha* occurred as tracheophytes became shrubby plants approximately 1 m tall^{28,29}. If *Rhyniognatha* had wings, for which we believe evidence is compelling, then the chronology for the evolution of wings would be more similar to that for arborescence.

The most definitive implication of *Rhyniognatha*'s dicondylid is that insects and hexapods as a whole must have originated during the Silurian period. Monocondylid insects must be older than *Rhyniognatha* (Fig. 2), and may have had an earlier start on terrestrial life than previously believed. Plants are now understood to have ventured onto land in the Ordovician period³⁰, and it is possible that amphibious invertebrates did so as well. Silurian terrestrial biotas probably included wingless insects as well as various chelicerate and myriapodous arthropods. Thus, the currently pervasive insects might not have been just the first organisms to take to the air with controlled flight (sometime during the Devonian period) but also may have been members of the earliest terrestrial faunas. □

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Diclofenac residues as the cause of vulture population decline in Pakistan

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The Oriental white-backed vulture (OWBV; *Gyps bengalensis*) was once one of the most common raptors in the Indian subcontinent¹. A population decline of >95%, starting in the 1990s, was first noted at Keoladeo National Park, India². Since then, catastrophic declines, also involving *Gyps indicus* and *Gyps tenuirostris*, have continued to be reported across the subcontinent³. Consequently these vultures are now listed as critically endangered by BirdLife International⁴. In 2000, the Peregrine Fund initiated its Asian Vulture Crisis Project with the Ornithological Society of Pakistan, establishing study sites at 16 OWBV colonies in the Kasur, Khanewal and Muzaffargarh–Layyah Districts of Pakistan to measure mortality at over 2,400 active nest sites⁵. Between 2000 and 2003, high annual adult and subadult mortality (5–86%) and resulting population declines (34–95%) (ref. 5 and M.G., manuscript in preparation) were associated with renal failure and visceral gout. Here, we provide results that directly correlate residues of the anti-inflammatory drug diclofenac with renal failure. Diclofenac residues and renal disease were reproduced experimentally in OWBVs by direct oral exposure and through feeding vultures diclofenac-treated livestock. We propose that residues of veterinary diclofenac are responsible for the OWBV decline.

Between 2000 and 2002 we performed gross post-mortem examinations on 259 adult and subadult OWBVs, of which 219 (85%) had grossly apparent urate deposits, characteristic of visceral gout, on the surface of internal organs (Fig. 1). Visceral gout in birds is most commonly the result of renal failure leading to hyperuricaemia and the deposition of uric acid on and within the internal organs, and can be caused by degenerative, metabolic, infectious, or toxic diseases⁶. To verify renal disease, and to determine the cause, detailed necropsies and diagnostic testing were performed on a subset of 42 OWBVs (33 adults and 9 juveniles; 28 with visceral gout and 14 without visceral gout) that were found within approximately 24 h of death. The remaining OWBVs were significantly decomposed and thus were unsuitable for diagnostic evaluation, although the grossly apparent and characteristic lesions of visceral gout allowed the presence or absence of the disease to be determined. Of the 14 OWBVs without visceral gout, we determined the cause of death in 8 (57%), which included trauma, intestinal foreign bodies, lead poisoning, organophosphate poisoning and gun-shot (Supplementary Information). We were unable to determine the cause of death in the remaining six vultures. Among the 28 OWBVs with visceral gout, only one (4%) had an identifiable disease (infection with *Mycobacterium avium*) in addition to visceral gout. All but two of the visceral gout cases were in good physical condition based on the subjective assessment of normal pectoral muscle mass and adequate body fat. In all of the OWBVs with visceral gout, the only significant histopathological lesion was severe, acute renal

Table 1 Diclofenac residue testing results in kidney samples from wild OWBVs with and without renal failure

Case no.	Date	Site	Age	Gout	Diagnosis	Diclofenac residues ($\mu\text{g g}^{-1}$)
33	2001	KS	Juv	Yes	None	0.051
74	2002	KH	Ad	Yes	None	0.054
16	2001	KS	Ad	Yes	None	0.060
53	2002	KH	Ad	Yes	None	0.064
60	2002	ML	Ad	Yes	None	0.064 (0.076)
39	2001	KH	Ad	Yes	None	0.077
69	2002	ML	Ad	Yes	None	0.079
57	2002	KH	Ad	Yes	None	0.080
40	2002	ML	Ad	Yes	None	0.091
20	2001	KS	Ad	Yes	None	0.097
15	2001	KS	Ad	Yes	None	0.099
35	2001	KS	Ad	Yes	None	0.106 (0.077)
41	2002	KH	Ad	Yes	None	0.109
75	2002	KH	Ad	Yes	None	0.114
55	2002	ML	Ad	Yes	None	0.124
54	2002	ML	Ad	Yes	None	0.177
71	2002	KH	Ad	Yes	None	0.179
61	2002	ML	Ad	Yes	None	0.186
42	2002	KH	Ad	Yes	None	0.199
44	2002	KH	Ad	Yes	None	0.233
4	2000	KS	Ad	Yes	<i>M. avium</i> infection	0.450
38	2001	ML	Ad	Yes	None	0.451
59	2002	ML	Ad	Yes	None	0.504
45	2002	ML	Ad	Yes	None	0.642 (0.197)
56	2002	KH	Ad	Yes	None	0.643
2	2000	LH	Ad	No	Wire collision	BDL
3	2000	CW	Juv	No	Hit by car	BDL
12	2001	ML	Ad	No	None	BDL
14	2001	KS	Ad	No	Lead poisoning	BDL
28	2001	KS	Ad	No	Normal (trapped)	BDL (BDL)
31	2001	KH	Juv	No	Fell from nest	BDL
46	2002	KH	Juv	No	Fractured tibia	BDL
47	2002	KH	Juv	No	Intestinal foreign body	BDL
49	2002	KF	Ad	No	None	BDL
50	2002	ML	Juv	No	None	BDL
51	2002	KH	Juv	No	None	BDL
52	2002	ML	Juv	No	None	BDL
58	2002	ML	Ad	No	Organophosphate	BDL (BDL)

BDL indicates 'below detection limit' of diclofenac assay (0.005–0.01 $\mu\text{g g}^{-1}$). Results in parentheses are from the Toxicology Laboratory at the University of Pennsylvania New Bolton Center, which were performed as independent verification. This additional testing also did not detect acetaminophen (0.05), flunixin (0.05), ibuprofen (0.50), phenylbutazone (0.10), oxyphenbutazone (0.05), indomethacin (0.05), ketoprofen (0.25), mefenamic acid (0.50), salicylic acid (1.0), tolmetin (0.05), or naproxen (1.0) (detection limits, in parentheses, are in $\mu\text{g g}^{-1}$). Ad, adult; CW, Chichawatni; Juv, juvenile; KF, Katora Forest; KH, Khanewal district study site; KS, Kasur district study site; LH, City of Lahore; ML, Muzaffargarh–Layyah district study site.