of the synthesized hybrids were considered *H. petiolaris* genomic regions (1), whereas linkage blocks lacking *H. petiolaris* markers were designated *H. annuus* regions (0), thereby generating the composite distribution of parental genomic regions shown in Fig. 2. Parental markers in *H. anomalus* either matched (0,0 or 1,1) or did not match (0,1 or 1,0) the corresponding genomic region in the experimental hybrids.

14. E. M. McCarthy, M. A. Amussen, W. W. Anderson, *Heredity* **74**, 502 (1995); V. Grant, *Genetics* **54**, 1189 (1966).
15. Spearman's rank correlation coefficient was calculated for each pairwise combination of the three hybrid lineages. The percentage at which a marker introgressed for a given lineage was scored in the following manner: 0.0% = 0; 1 to 25% = 1; 26 to 50% = 2; 51 to 100% = 3. Lineages I × II, $r_s = 0.79$, $P < 0.0001$; lineages I × III, $r_s = 0.74$, $P < 0.0001$; lineages II × III, $r_s = 0.73$, $P < 0.0001$.
16. N. H. Barton and G. M. Hewitt, *Heredity* **47**, 367 (1981).
17. C.-I. Wu and M. F. Palopoli, *Annu. Rev. Genet.* **27**, 283 (1994).
18. The following equation was used to compute the test statistic (ρ) for two-way epistatic interactions among unlinked loci (N_{loci}, number of unlinked loci; $N_{progeny}$, number of progeny tested; and $locus_{n,p} = 0$ if the *H. petiolaris* marker is absent and 1 if present):

$$\rho_{i\times j} = \frac{\sum_{p=1}^{N_{progeny}} \prod_{n=i,j} locus_{n,p}}{\sqrt{\sum_{p=1}^{N_{progeny}} locus_{i,p} \times \sum_{p=1}^{N_{progeny}} locus_{j,p}}}$$

where $0 \le \rho_{i\times j} \le 1$ (1)

Equation (1) can be generalized to *N*-way epistatic interactions:

$$\rho_{1\times\ldots\times N_{loci}} = \frac{\sum_{p=1}^{N_{progeny}} \prod_{n=1}^{N_{loci}} locus_{n,p}}{N_{loci}\sqrt{\prod_{n=1}^{N_{loci}} \sum_{p=1}^{N_{progeny}} locus_{n,p}}}$$

where $0 \le \rho_{i\times j} \le 1$ (2)

Significance for each two- or three-way association was tested by comparing $\rho_{observed}$ with $\rho_{expected}$ as computed by bootstrap randomization of the observed data (N = 10,000 per association) (Fig. 3).
19. Seventy-four percent of epistatic markers were found in all three lineages in contrast to 10% of nonepistatic markers ($G^2 = 30.6$, df = 2, $P < 0.0001$). However, part of this correlation may be due to the greater power of the ρ test statistic in detecting associations among markers of intermediate frequency.
20. L. H. Rieseberg, A. M. Desrochers, S. J. Youn, *Am. J. Bot.* **82**, 515 (1995).
21. Positive two-way associations occur when unlinked *H. petiolaris* markers appear together within individuals of the progeny array more often than would be expected by chance, suggesting nonadditive, positive fitness effects (for example, increased pollen viability) when these markers appear together. By contrast, negative associations occur when unlinked *H. petiolaris* markers appear together less often than would be expected by chance, suggesting nonadditive negative fitness effects when these markers appear together.
22. Positive three-way associations are similar to two-way associations (*21*), except that three *H. petiolaris* markers are involved. Negative three-way associations may consist of negative two-way associations only or a combination of negative and positive two-way associations.

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TECHNICAL COMMENTS

Origin of Replication of *Mycoplasma genitalium*

The complete sequence of the genome of *Mycoplasma genitalium* was reported and analyzed by Claire M. Fraser *et al.* (*1*). The origin of replication of the chromosome was not localized precisely because of the lack of consensus patterns, "DnaA boxes," in this species, but it was suggested that the origin might be in an untranscribed AT-rich region between *dnaA* and *dnaN*. This location can be confirmed using a new method based on the results of the mathematical analysis of the model of DNA evolution under no–strand-bias conditions (*2*)—that is, when there is no strand-bias for the mutation process or for the selective process between the two strands of DNA.

Under no–strand-bias conditions, the equilibrium point is such that the base frequencies in each strand always respect [A]=[T] and [C]=[G] equalities, regardless of the initial state of the DNA sequence and of details of the substitution patterns. Any significant deviation from the intra-strand rules [A]=[T] or [C]=[G] is an indication that there is an inequality in the substitution patterns between the two strands of DNA. The null hypothesis for the detection of such an inequality is then the appearance of intra-strand equifrequencies [A]=[T] and [C]=[G]. Because the mechanisms for DNA replication differ between the leading strand and the lagging strand (*3*), at least in vitro, mutation patterns could differ depending on

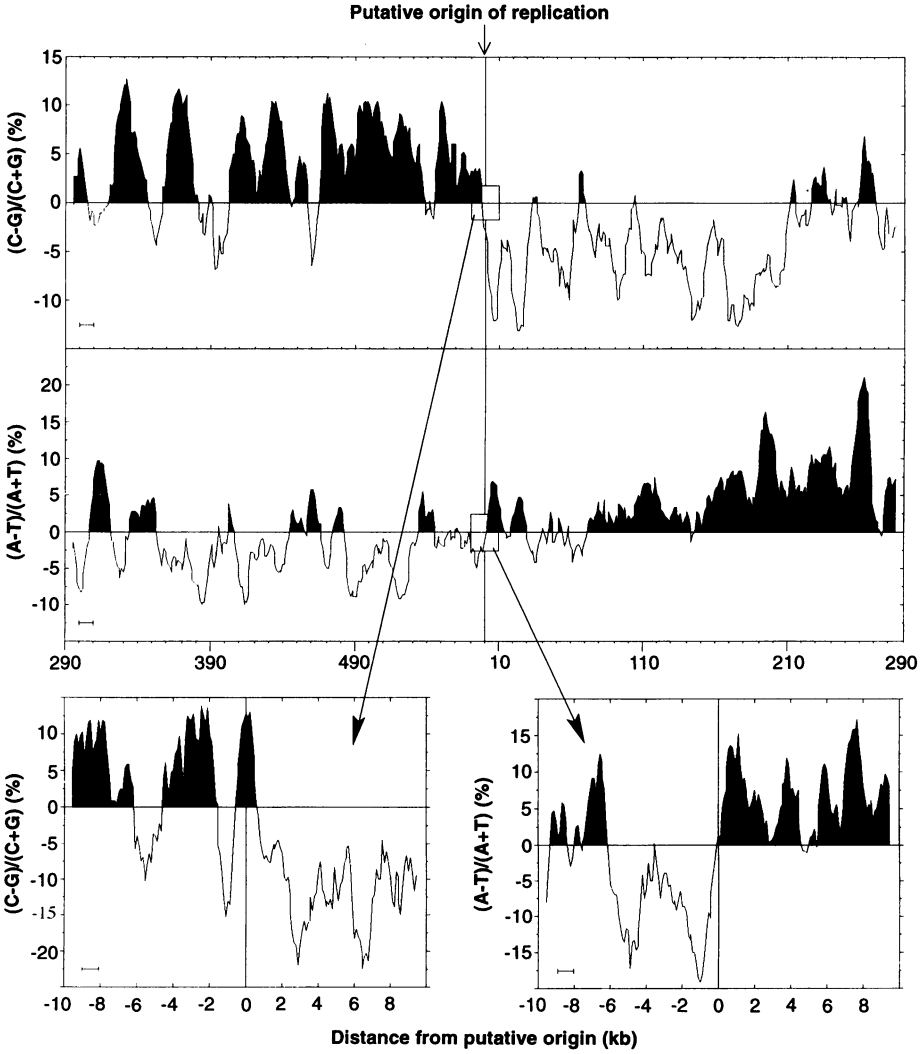

Fig. 1. Origin of replication in *M. genitalium*, showing switch of polarity of base composition asymmetries. Moving window size and step: top 10 kb, 1 kb; bottom 1 kb, 0.1 kb. Each data point is at the middle of its window.

which strand is being copied, so that a rejection of the null hypothesis is expected, yielding an asymmetry in [A]=[T] or [C]=[G] equifrequencies. This asymmetry is expected to switch polarity at the origin and terminus of replication of the chromosome and does switch polarity at the origin and terminus of replication in *Escherichia coli*, *Bacillus subtilis*, and *Haemophilus influenzae*, splitting the circular chromosome into two chirochores (*4*).

In *Mycoplasma genitalium*, the new method clearly confirms the suggested origin of replication (Fig. 1). The asymmetry in base composition, A-T, is −2.0% ± 0.4 before the origin of replication and +4.5% ± 0.4 thereafter; and the asymmetry in base composition, C-G, is +4.0% ± 0.7 before the origin of replication and −4.5% ± 0.6 thereafter. The switch of the polarity of base composition asymmetries is then significant. This method for the detection of replication origins is useful for the analysis of a new genome when the consensus pattern approach fails.

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Classification of the Arthropod *Fuxianhuia*

Jun-yuan Chen *et al.* (*1*) provided an invaluable description of *Fuxianhuia*, a problematic arthropod from the celebrated Chinese Chengjiang fauna (*2*). Pointing to the apparently rudimentary nature of the protocephalon and the unspecialized morphology of the trunk appendages, they classified *Fuxianhuia* as a basal euarthropod, a primitive and generalized representative of the phylum. They did not, however, test their assertion with the necessary cladistic analysis. Such an analysis reveals that *Fuxianhuia* is an arachnomorph (*3–5*), specifically grouping with the chelicerates (arachnids and merostomes). This result establishes *Fuxianhuia* as the earliest known chelicerate by as much as 60 million years.

I coded the new information from Chen *et al.* into a growing database that has been found effective for investigating higher order relationships among Cambrian and Recent arthropods (*3*, *4*). A reduced set of taxa were analyzed using the program PAUP 3.1.1 (Smithsonian Institution, Washington, DC), which produced gross topology consistent with that derived from previous analyses (*3*, *6*). *Fuxianhuia* consistently grouped with the chelicerates (despite the absence of chelicerae and minimal cephalizaton) in the larger clade of arachnomorphs. To investigate this relationship more fully, a further run was made, reinstating the entire arachnomorph clade (*3*) and incorporating data from the Silurian eurypterid *Baltoeurypterus* (*7*), the Devonian xiphosuran *Weinbergina* (*8*), and the problematic *Cheloniellon* (*9*, *10*) (Fig. 1). *Fuxianhuia* consistently fell within the chelicerates, with *Cheloniellon* and *Aglaspis* basal to them. The arrangement of several arachnomorphs was modified over some previous analyses (*3*), demonstrating the potentially pivotal role of fossils exemplifying new character state combinations (*11*).

As noted by Chen *et al.* (*1*), *Fuxianhuia* displays a number of characters typical of more basal arthropods. However, caution is required when constructing a model solely on the purportedly directional evolution of small numbers of characters (*12*). The presence of numerous trunk somites bearing relatively undifferentiated appendages is traditionally considered a plesiomorphic feature, but recent work on homeobox genes indicates that this may be an unwarranted assumption (*13*). Multiarticulate endopods are often regarded as primitive but are also known from derived (*3*, *4*) groups of atelocerates (*14*), crustaceans (*15*), and other arachnomorphs (*16*). The significance of diplosegmentation is as yet unclear (*17*, *18*). Thus, these characters do not oppose a chelicerate affinity.

Fuxianhuia also exhibits several features, however, that make it unique among chelicerates. Given the early plasticity of arthropod bodyplans (*3*, *19*, *20*), these differences may be expected, but their occurrence in a taxon in this location is worthy of comment. The anteriormost appendages are multiarticulate antennae rather than chelicerae. The absence of chelicerae was used to exclude the aglaspids from the chelicerates *sensu stricto* (*21*), but the two groups show many derived similarities (Fig. 1) and are probably closely related (*22*). Similar considerations apply to *Fuxianhuia*. Al-

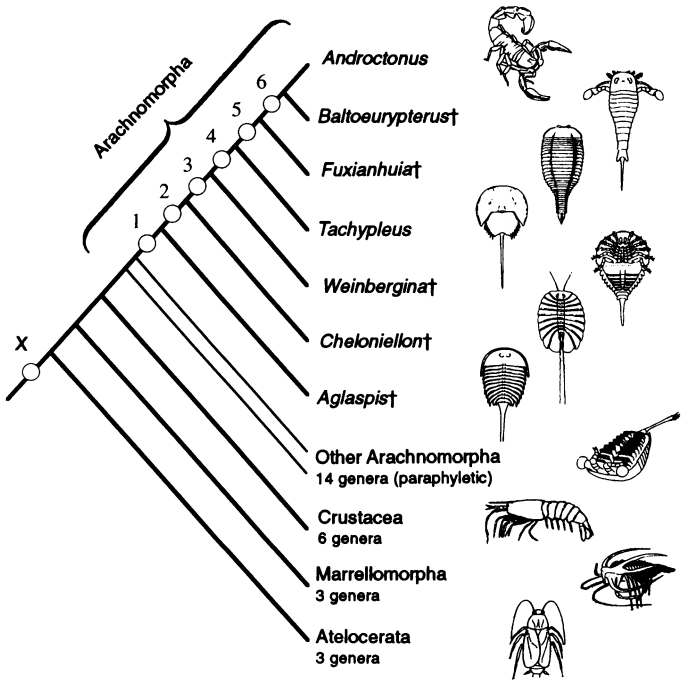

Fig. 1. Simplified cladogram of Recent and fossil euarthropod taxa (strict consensus). Detailed topology illustrated for chelicerates and allied genera. †, Fossil genus. X, Location of *Fuxianhuia* proposed by Chen *et al.* (*1*). Shared, derived characters are (DELTRAN optimization): (i) Dorsal cuticle trilobed. Cuticle strongly tuberculate. Fourteen somites in cephalon and trunk. Exopod of second appendage absent. Inner rami of trunk appendages composed of four podomeres and lacking spines. (ii) Six appendages in the cephalon. Labrum detached. Gnathobases absent from trunk appendages. (iii) First appendages chelicerae. Cephalic shield with marginal ecdysial sutures. Cardiac lobe present. Trunk tergites with straight posterior margin in dorsal aspect. (iv) Cephalic shield with marginal rim. Postcephalic articulation without overlapping pleurae. Two median eyes. Outer rami of trunk appendages lacking fine filaments (rounded and lamellate). Cuticle smooth. (v) Trunk tagmatized (mesosoma and metasoma in eurypterids and arachnids). (vi) Dorsal cuticle not trilobed. Inner and outer rami of trunk appendages absent. Eighteen somites in the cephalon and trunk.

Origin of Replication of Mycoplasma genitalium

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