

EVOLUTIONARY BIOLOGY

Evolutionary trade-off in reproduction of Cambrian arthropods

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Trade-offs play a crucial role in the evolution of life-history strategies of extant organisms by shaping traits such as growth pattern, reproductive investment, and lifespan. One important trade-off is between offspring number and energy (nutrition, parental care, etc.) allocated to individual offspring. Exceptional Cambrian fossils allowed us to trace the earliest evidence of trade-offs in arthropod reproduction. †*Chuandianella ovata*, from the early Cambrian Chengjiang biota of China, brooded numerous (≤ 100 per clutch), small (\emptyset , ~ 0.5 mm) eggs under carapace flaps. The closely related †*Waptia fieldensis*, from the middle Cambrian Burgess Shale of Canada, also brooded young, but carried fewer (≤ 26 per clutch), larger (\emptyset , ~ 2.0 mm) eggs. The notable differences in clutch/egg sizes between these two species suggest an evolutionary trade-off between quantity and quality of offspring. The shift toward fewer, larger eggs might be an adaptive response to marine ecosystem changes through the early-middle Cambrian. We hypothesize that reproductive trade-offs might have facilitated the evolutionary success of early arthropods.

INTRODUCTION

A biological trade-off is generally considered as a condition in which an increase in the performance of one trait causes a decrease in the performance of another, given the limited amount of available resource (energy, time, space, etc.) (1). Parents of most animals [including humans (2)] are usually confronted with a trade-off between investments in quantity and quality of offspring. To invest more in quality, parents lower the quantity of offspring and exert more energy to augment the survival prospects of their progeny by protecting them from predation, parasitism, food shortage, and unfavorable environmental factors (3). As a widespread life-history trait among animal groups, parental care encompasses basic characteristics such as protecting embryos until they hatch and more complex behaviors such as carrying, guarding, shepherding, grooming, and sharing food with the young (4). Brooding and carrying prehatched eggs by adult females are especially frequent in modern pancrustacean groups (5), particularly Ostracoda (6), Branchiopoda (7), Copepoda (8), and Malacostraca (9). This basic form of brood care arose early in arthropod evolution, as evidenced by the fossilized eggs of †*Kunmingella douvillei* (10), an ostracod-like arthropod from the early Cambrian Chengjiang biota, and fossilized embryos of †*Waptia fieldensis* (11), a “bivalve” arthropod from the middle Cambrian Burgess Shale.

Forms closely related to †*W. fieldensis* are known from the early Cambrian of China [e.g., †*Chuandianella ovata* (12), †*Synophalos xynus* (13), and probably †*Clypeacaris* spp. (14)] and Greenland [†*Paulotermis spinodorsalis* (15)]. Among them, †*C. ovata* from the Chengjiang

biota was first described as a bradoriid species under †*Mononotella* (16), then assigned to a new genus †*Chuandianella* (17), and later referred to †*Waptia* (18–20). Most recently, †*C. ovata* was reevaluated and restored to the genus †*Chuandianella* (12), but many key aspects of its anatomy and life-history traits require further investigation. We describe here exceptionally preserved specimens of †*C. ovata*, which carried a relatively large number of small eggs, in contrast to the evolutionarily younger †*W. fieldensis* (11), which brooded much fewer but larger eggs than †*C. ovata*. The two negatively correlated traits (i.e., number versus size of eggs) exhibited in the Cambrian waptiids are viewed here as evidence for an evolutionary trade-off in reproduction of early pancrustacean arthropods. These findings shed light on the evolution of life-history strategies in early animals.

RESULTS

Comparative anatomy of †*C. ovata* and †*W. fieldensis*

†*C. ovata* (16) from the Chengjiang biota (Cambrian Series 2, stage 3; ~ 520 Ma ago; South China) is a shrimp-like arthropod generally measuring 2 to 3 cm in adult body length (see the Supplementary Materials for details on taphonomy and preservation). †*C. ovata* is characterized by a bivalved, ovate carapace covering most of its cephalothoracic segments, a pair of long antennules, stalked eyes, and four pairs of post-antennular uniramous appendages followed by six pairs of lamellate appendages. Its abdominal region consists of six limbless cylindrical segments and is terminated by a tail fan with two three-segmented lateral flaps (Fig. 1, A, C, E, G, H, and J, and fig. S1). Although smaller in adult size, †*C. ovata* notably resembles †*W. fieldensis* (21) from the middle Cambrian Burgess Shale (Miaolingian, Wuliuan; ~ 508 Ma ago; Canada) in most aspects of its overall morphology, tagmosis, and appendage structure (Fig. 1, B, D, F, I, and K, and table S1). A recent in-depth exploration of this iconic Burgess Shale arthropod revealed its mandibulate affinities and supports its position as a stem-group pancrustacean (22) assigned to Hymenocarina (22, 23). Our detailed comparison (table S1) and phylogenetic analysis (fig. S2) underpin a close relationship of these two Cambrian waptiid species (see the Supplementary Materials for systematic paleontology of waptiid arthropods).

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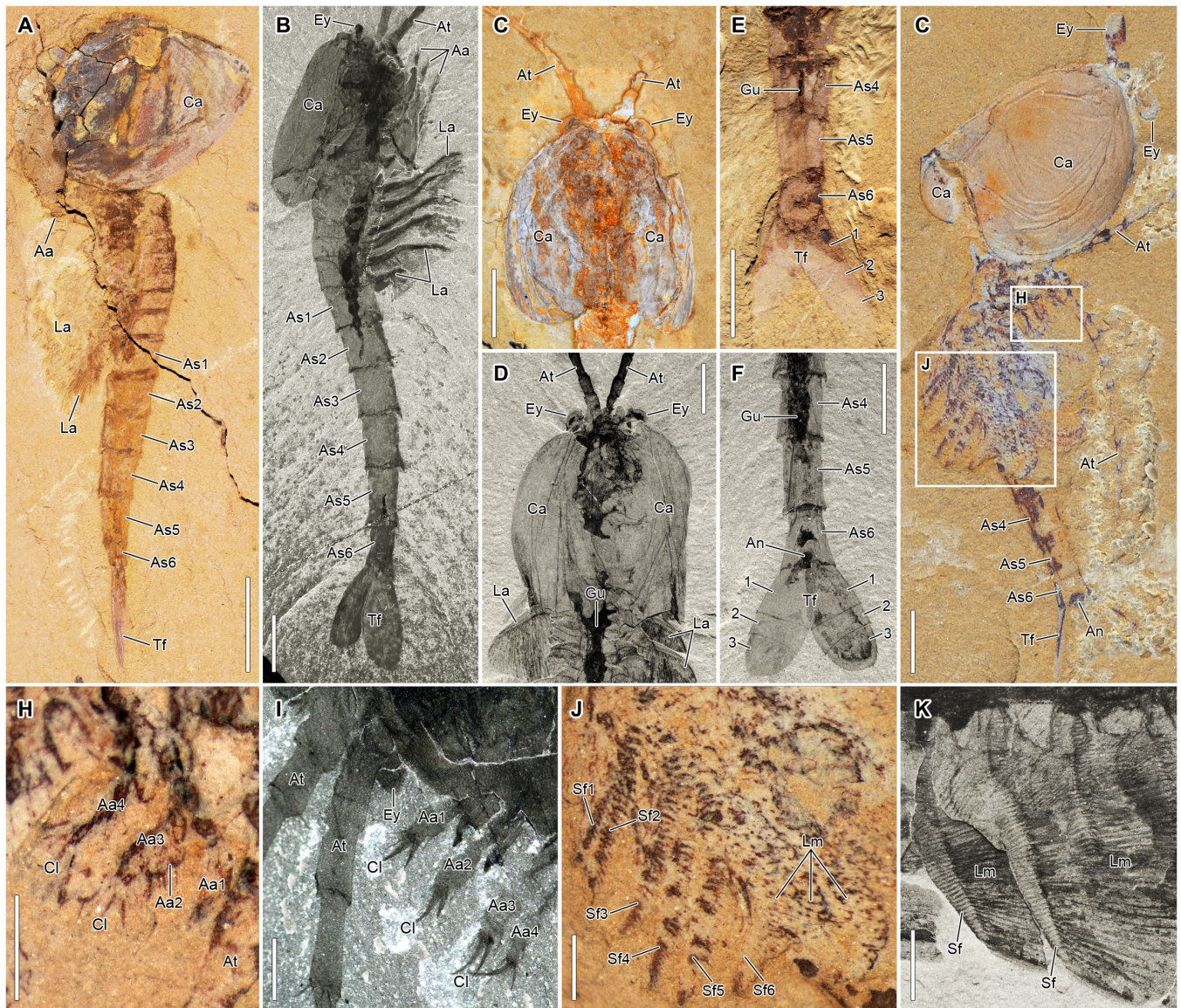


Fig. 1. Comparative morphology of †*C. ovata* (early Cambrian, Chengjiang) and †*W. fieldensis* (middle Cambrian, Burgess Shale). (A and B) Complete specimen of †*C. ovata* (YKLP14400) and †*W. fieldensis* (USNM 114259) showing general morphology in lateral view. (C and D) Anterior part of †*C. ovata* (YKLP14401) and †*W. fieldensis* (USNM 138231) in dorsal view. (E and F) Posterior part of †*C. ovata* (YKLP14402) and †*W. fieldensis* (USNM 138231) with a tripartite tail fan (segments numbered). (G) Oblique ventral view of †*C. ovata* (YKLP14403) showing a dislocated carapace, anterior uniramous appendages [small box, see (H)], and posterior lamellate appendages [large box, see (J)]. (H and I) Details of uniramous appendages in †*C. ovata* and †*W. fieldensis* (ROMIP 64281). (J and K) Details of lamellate appendages in †*C. ovata* (six on the right-hand side labeled) and †*W. fieldensis* (USNM 275504), each composed of an annulated shaft fringed with lamellae. Aa, anterior (uniramous) appendage; An, anus; As, abdominal segment; At, antennule; Ca, carapace; Cl, claws; Ey, eye; La, (posterior) lamellate appendage; Lm, lamellae; Gu, gut; Sf, shaft of lamellate appendage; Tf, tail fan. Scale bars, 2 mm (A, G, and K), 5 mm (B to F), and 1 mm (H to J). Photo credit: Jean Vannier, Université Lyon 1 (A to K).

Egg clusters in †*C. ovata*

Only 4 of 1020 examined specimens of †*C. ovata* show clustered, rounded to ovoid structures nested under the lateral flaps of the carapace (Figs. 2 and 3, figs. S3 to S7, and Table 1). The clusters have a consistent dorso-posterior location within the interspace between the body and the inner surface of the carapace (fig. S6). They are closely packed, rounded structures of approximately the same size, highly reminiscent of the brooded eggs of extant and fossil bivalved crustaceans [e.g., ostracods (24–26) and spinicaudatans (25, 27)] and the middle Cambrian waptiid †*W. fieldensis* (11, 22) (fig. S8).

The best-preserved clusters contain 50, 38, and 36 eggs (Figs. 2 and 3), whereas an incomplete carapace shows ~18 poorly preserved eggs (fig. S7). The diameter of eggs shows very little variation within individual clusters and between the four specimens, with an average size of 0.49, 0.52, 0.53, and 0.55 mm, respectively (Table 1). The area occupied by the eggs ranges from 13 to 21% of the carapace surface area. The clutch size of ELEL-SJ081254 is most likely underestimated owing to organic stains that obscure the upper left part of the egg cluster (Fig. 2, A to C). The carapace length and body size of this specimen are almost identical to that of ELEL-MF140101

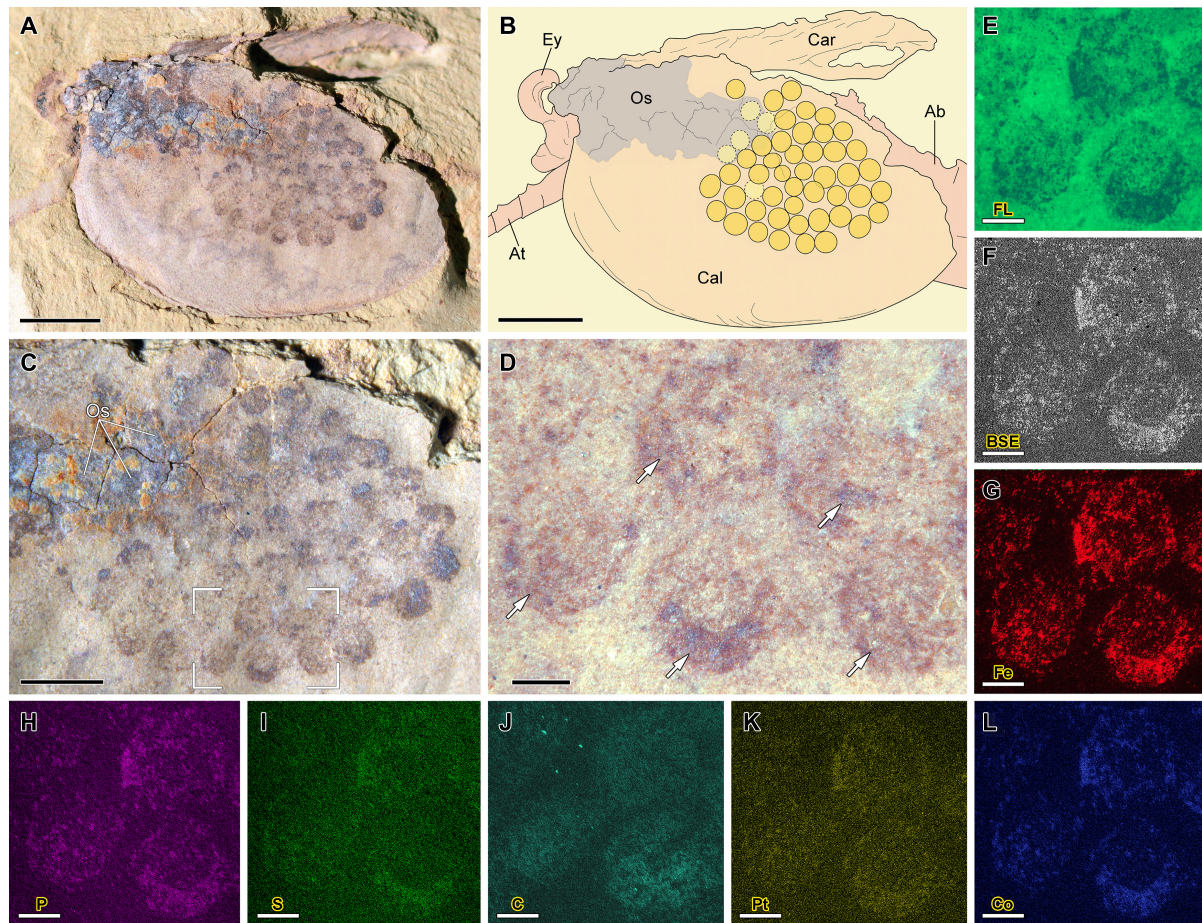


Fig. 2. Egg clusters in †C. ovata. (A) Laterally compressed specimen (ELEL-SJ081254A) showing a cluster of eggs under its left carapace valve. (B) Interpretative drawing of (A) illustrating largely hexagonal packing of eggs; poorly preserved eggs represented by dashed circles. (C) Detail of egg cluster. (D) Close-up of focus area in (C); arrows indicate possible remains of embryonic tissues. (E to L) Images of left three eggs in (D) obtained by fluorescence (FL) microscopy, backscattered electron (BSE) microscopy, and energy-dispersive x-ray spectroscopy (EDX; Fe, P, S, C, Pt, and Co), respectively. Ab, abdomen; At, antennule; Cal, left valve of carapace; Car, right valve of carapace; Ey, eye; Os, organic stains. Scale bars, 2 mm (A and B), 1 mm (C), and 200 μ m (D to L). Photo credit: Qiang Ou, China University of Geosciences, Beijing (A, C, and D).

Table 1. Measurements of ovigerous specimens of †C. ovata and †W. fieldensis. Abbreviations: Ave D, average diameter of eggs; Bl, body length (measured from the anterior end of carapace to the posterior end of tail fan); Ch, carapace height; Cl, carapace length; Cs, carapace surface area (one valve); Es, carapace surface area covered by preserved eggs; Es/Cs, ratio of Es to Cs; Max D, maximum diameter of eggs; Min D, minimal diameter of eggs; N, number of eggs preserved; *, extrapolated measurement.

Specimen	Section	N	Min D (mm)	Max D (mm)	Ave D (mm)	Bl (mm)	Cl (mm)	Ch (mm)	Es (mm ²)	Cs (mm ²)	Es/Cs (%)
†C. ovata											
ELEL-SJ081254	Sanjiezi	50	0.405	0.588	0.520	22.50	9.88	6.56	10.62	50.95	20.84
ELEL-MF140101	Mafang	38	0.435	0.617	0.554	22.85	9.80	6.40	7.51	46.26	16.23
YRCP 0010	Ercaicun	36	0.400	0.550	0.489	23.00	9.60	6.66	6.76	50.67	13.34
ELEL-EJ081702	Erjie	>18	0.443	0.606	0.530	27*	12*	7.85	>4.0	69*	>5.7
†W. fieldensis (11)											
ROMIP 63357	Walcott Quarry	13	2.25	2.62	2.4	63.7	20.7	?	58.8	288*	20.4
ROMIP 63354	Walcott Quarry	12	2.06	2.36	2.18	60*	19.6	17.0	44.8	280	16.0

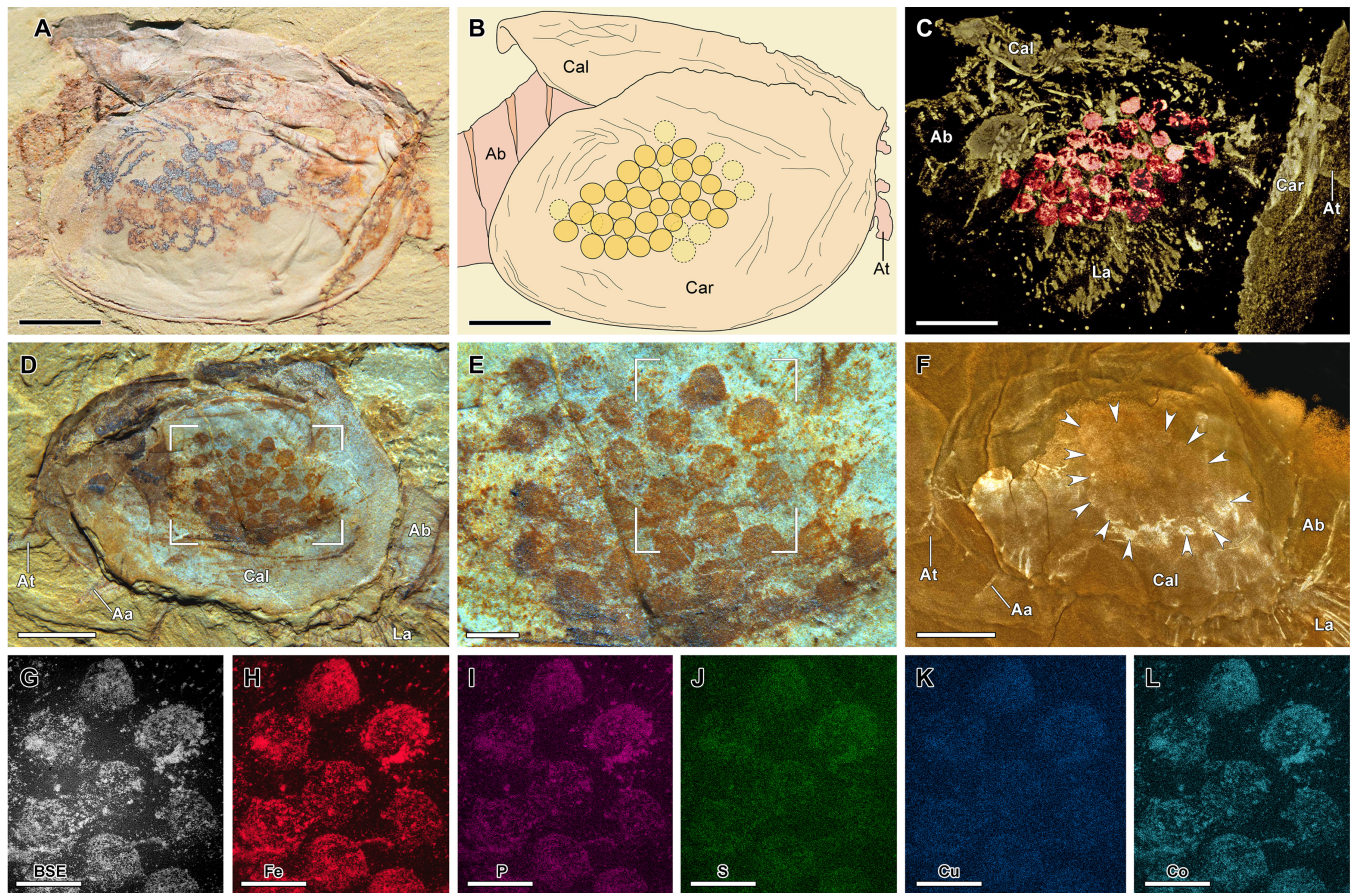


Fig. 3. Egg clusters in †*C. ovata*. (A) Oblique-laterally compressed specimen (ELEL-MF140101A) with eggs under its right carapace valve. (B) Interpretative drawing of (A); poorly preserved eggs represented by dashed circles. (C) Volume rendering from micro-computed tomography (micro-CT) data of (A); eggs highlighted in red. (D) Laterally compacted specimen (YRCP 0010) with eggs. (E) Detail of egg cluster in (D). (F) Volume rendering from micro-CT data of (D); arrowheads indicate eggs. (G to L) Images of hexagonally arranged eggs [focus area in (E)] obtained by BSE microscopy and EDX (Fe, P, S, Cu, and Co), respectively. Aa, anterior (uniramous) appendage; Ab, abdomen; At, antennule; Cal, left valve of carapace; Car, right valve of carapace; La, lamellate appendage. Scale bars, 2 mm (A to D and F) and 500 μm (E and G to L). Photo credit: Qiang Ou, China University of Geosciences, Beijing (A); Ailin Chen, Yuxi Normal University (D and E).

and YRCP 0010 (Table 1), but the latter two show fewer eggs (Fig. 3, A to F).

The egg clusters of †*C. ovata* are positioned on either the left (ELEL-SJ081254 in Fig. 2A and YRCP0010 in Fig. 3D) or the right (ELEL-MF140101 in Fig. 3A and ELEL-EJ081702 in fig. S7A) valve of the carapace, suggesting that the eggs were deposited and brooded bilaterally on both sides of the body. No features indicate that the eggs were attached via a stalk to the body or the carapace or that the clusters were contained within a sac-like structure. The clusters have an overall elliptical outline, with the major axis accounting for about half the length of the carapace (Figs. 2, A to C, and 3, A to F). Each cluster seemingly consists of a single layer of eggs, as inferred from no or very limited overlap among the eggs. The eggs are largely in contact with one another and arranged in staggered rows (e.g., ELEL-SJ081254 in Fig. 2, A to D), indicating a hexagonal close-packing arrangement (Fig. 3, G to L).

The eggs of †*C. ovata* are largely preserved as iron oxides, rendering them a typical reddish to dark purple color. In contrast to the surrounding matrix and the carapace, the eggs are highly enriched in iron (Figs. 2G and 3H), as indicated by energy-dispersive x-ray spectroscopy (EDX). Iron oxide is the weathering product of au-

thigenic pyrite, which precipitated during the early stages of decay via microbial sulfate reduction in anoxic conditions (28). This process is generally invoked to explain the preservation of soft-bodied or slightly sclerotized Chengjiang animals (29). Slight elevation in phosphorus, sulfur, carbon, cobalt, platinum, and copper is detected within the eggs (Figs. 2, H to L, and 3, I to L, and figs. S3 to S5). Green light fluorescence (FL) and backscattered electron (BSE) images reveal the external boundaries of eggs with higher accuracy and also possible internal features (e.g., Fig. 2, E and F, and fig. S4, D and E). A few eggs show crescent-shaped contents (Fig. 2, D to L) and some impart a segmented appearance (e.g., fig. S3B), which recalls ventrally flexed embryos with segmental rudiments in some bivalved crustaceans such as phyllocarids (30). Other irregular or more diffuse patches of iron oxides may represent decayed internal tissues or have resulted from weathering or diagenetic processes. X-ray micro-CT imaging reveals three-dimensional morphology of the eggs as thin discoid structures (after compaction), which are distributed in a region not corresponding to the appendages (Fig. 3, C and F) and which seemingly lie closer to the carapace than to the body (fig. S6). The buried flap of the carapace and underlying eggs, due to poor pyritization, were not detected in the specimens via micro-CT.

None of the imaging techniques used here revealed the presence of a thick external wall around the eggs comparable with the egg capsule of extant clam shrimps (Spinicaudata) (31).

DISCUSSION

Brood care in the Chengjiang waptiid †*C. ovata*

Females of the majority of extant crustaceans brood their fertilized or parthenogenetic eggs until they hatch or release them after a brooding period. The brooding mode of †*C. ovata* can be best compared with that of modern bivalved crustaceans such as spinicaudatan, laevicaudatan, and cyclestherid branchiopods (7) (fig. S9, A to E), myodocope ostracods (7) (figs. S9, F to H, and S10), and thermosbaenacean peracarids (32) in which embryos are accommodated dorsally or posteriorly within a chamber between the female's body and carapace. By contrast, most peracarids, anostracans, and notostracans carry their eggs within a ventral brood pouch, whereas cladocerans brood their eggs in a dorsal brood pouch between the carapace and the dorsum of the trunk (33). Embryos of phyllocarids (e.g., *Nebalia bipes*) are kept within a brood basket constituted by a setose network of endopods (34). Brooded embryos of extant crustaceans may be released before hatching, as exemplified by dormant or resting eggs of some branchiopods (35). Brood care occurs in fossil crustaceans such as branchiopods (including members of Anostraca, Notostraca, and Spinicaudata) of Devonian and Carboniferous (25, 27), and ostracods of Ordovician, Silurian, and Carboniferous (24–26), as well as in the Cambrian pancrustacean †*W. fieldensis* (11).

We show here that the presumed females of the early Cambrian waptiid †*C. ovata* also brooded their eggs under the lateral flaps of carapace. This location might have provided the eggs with optimal protection against physical damage and predators and might have limited parasite or fungal infestation. The movement of lamellate appendages (Fig. 1J) involved in both swimming and gas exchange [as seen in †*W. fieldensis* (22)] might also have generated a current between the body and the carapace to ensure ventilation over egg clusters. The three specimens of †*C. ovata* with well-preserved eggs are almost identical in body size (Figs. 2 and 3), but the egg number in ELEL-SJ081254 is higher than that in ELEL-MF140101 and YRCP 0010 (Table 1). This could be a result of egg loss/mortality during development or due to a sudden burial of the ovigerous female before the clutch reached its full size. Other reasons, such as individual variation or seasonal influence, might also be responsible for the difference in egg numbers.

The dorsolateral concentration of eggs under the carapace in †*C. ovata* is reminiscent of the brooding mode of modern clam shrimps (fig. S9, A to E), in which eggs are emitted through paired oviduct openings located in a particular trunk segment and spread out within the interspace between the carapace and the body, with specialized oviduct secretions facilitating eggs to form a cohesive cluster. The compact arrangement of eggs and the absence of ovigerous stalks or sacs in †*C. ovata* suggest that the eggs were stuck together probably by a mucous substance secreted by the female and that they were arranged in a single layer, most likely adhering to the inner wall of the carapace, as suggested by the micro-CT data (fig. S6E). The gonopores of †*C. ovata* are inferred to be located at the base of a specific pair of thoracic appendages, presumably corresponding to the center of the clusters. However, the eggs of extant clam shrimps (except cyclestherids) are typically released by the female as resting eggs after a brief brooding period. By comparison, the eggs of †*C. ovata* lack

characteristic features of such resting eggs (e.g., a thick, ornamented eggshell) and might have been brooded over a longer period by the female before being released as larvae or juveniles, as in modern myodocope ostracods (7).

The remarkably consistent size of eggs in †*C. ovata* suggests that each cluster corresponds to a single clutch of embryos and a synchronous embryonic stage. The crescent-shaped regions with segmented features observed in some eggs (Fig. 2, D to L, and fig. S3D) indicate an early stage of development in which the embryo occupied only part of the egg volume. This is supported by concentration of phosphorus in the crescent regions (Fig. 2H), as embryos of extant crustaceans are richer in phosphorus than surrounding yolk (36). The eggs of †*C. ovata* probably had a very thin, flexible outer membrane as indicated by the slight variations of their external shape from round to elliptic. In this respect, they are more comparable with the brooded embryos of myodocope ostracods than with the thick-walled resting eggs of branchiopods (figs. S9 and S10).

In summary, the egg clusters of †*C. ovata* represent the earliest (~520 Ma ago) known records of brood care using the carapace flaps. By contrast, the coeval bivalved bradoriid arthropod †*K. douvillei* (10) carried the eggs using three pairs of posterior appendages. By carrying the eggs under the carapace, †*C. ovata* might have provided the progeny with optimal protection and liberated the limbs that could be used otherwise. The compact arrangement and bilateral disposition of eggs in †*C. ovata* suggest that eggs were emitted through paired gonopores and that the bivalved exoskeleton played a key role in protection and incubation of eggs. This mode of reproduction and parental care might have remained virtually unchanged since the early Cambrian, as seen in various modern analogs such as branchiopods and ostracods (figs. S9 and S10).

Evolutionary trade-off in reproduction of Cambrian waptiids

†*W. fieldensis* and †*C. ovata* share numerous morphological traits and are closely related waptiid species, despite their different geological age, geographic distribution, and body size. Adult and subadult specimens of †*C. ovata* are typically 20 to 30 mm long (12), whereas those of †*W. fieldensis* are approximately double the size (40 to 60 mm) (22). The brooded egg mass occupies a similar proportion (ca. 20%) of their carapace surface (Es/Cs) in both species (Table 1), which might correspond to their maximum brooding capacity. In addition, both species kept the eggs in a similar position under the carapace. However, major differences occur in their clutch size and egg dimension (Fig. 4). †*W. fieldensis* had a small clutch size of up to 24 eggs with a large mean diameter varying from 1.35 to 2.4 mm in well-preserved specimens (fig. S8) (11). The poor preservation and embedding condition of eggs in ROMIP 63355 (fig. S8, E and F) do not allow accurate measurements; hence, the assumed smaller egg size (11) of this specimen remains questionable and is likely an artifact due to postmortem shrinkage, detachment from and hence burial deeper than the carapace, or other taphonomic effects. In extant crustaceans, the egg size of closely related species usually shows no notable variation. For example, the eggs of a closely related group of myodocope ostracods that live in similar marine habitats have virtually the same size (fig. S10, A to F). In addition, the size variation from a fertilized egg to an embryo during development of living bivalved crustaceans (e.g., myodocope ostracods; fig. S10, G to J) is generally insignificant (37), albeit in few decapods the size may increase up to 40% by osmotic uptake of water (38). By contrast, the clutch of †*C. ovata* contained four times as many (up to 100)

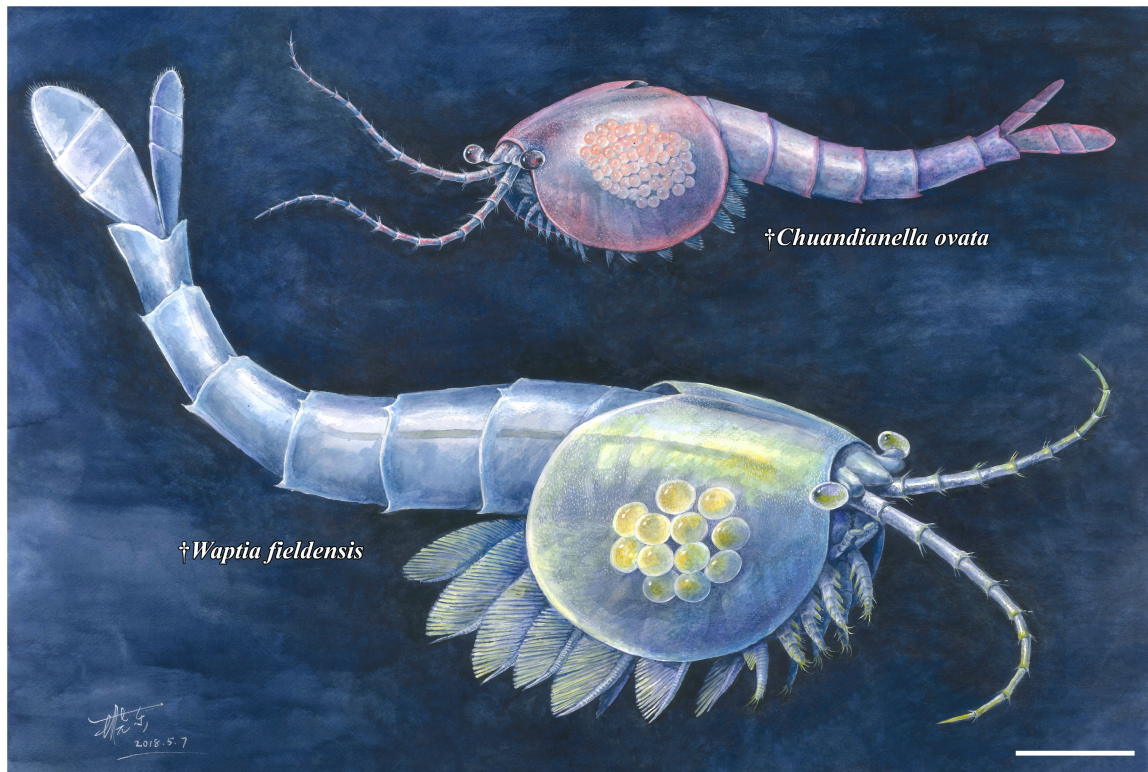


Fig. 4. Artistic reconstruction of the waptiids †*C. ovata* and †*W. fieldensis* with brooded eggs. Scale bar, 5 mm (for both species). Artwork credit: X. D. Wang.

eggs with a mean diameter ($\varnothing \sim 0.5$ mm) about one-fifth to one-third that of †*W. fieldensis* (Table 1).

Our findings identify two distinct reproductive strategies in waptiids: females of †*C. ovata* brooded a relatively high number of small eggs, whereas those of †*W. fieldensis* carried a low number of large eggs (Fig. 4). Small eggs and large clutch size might have allowed †*C. ovata* to maximize fecundity while investing less resource in each progeny. In comparison, the production of large and yolky eggs in †*W. fieldensis* might have augmented the fitness of individual offspring. Larger eggs generally supply offspring with greater nutrient reserves during development and are beneficial to juvenile growth and survival in response to adverse environmental factors, such as limited food or high predation risk (39). Hence, by yielding larger eggs and probably brooding them over a longer period, †*W. fieldensis* increased the parental care and the probability for individual offspring to survive to adulthood. Furthermore, production of larger eggs necessarily involved a decrease in clutch size in †*W. fieldensis* under the resource acquisition-allocation model (or the “Y model”), which posits that two traits cannot be simultaneously enhanced for a given amount of resource (40). The negatively correlated brooding traits in these two Cambrian waptiids might imply an evolutionary trade-off between quantity and quality of offspring (fig. S11).

Ecological and evolutionary implications

The factors that drove the shift in reproductive tactics of the two Cambrian waptiid arthropods remain speculative. The determinants of optimal investment (allocation of limiting resources) between clutch size and egg size have been a subject of debate even for extant organisms (41). Divergent reproductive strategies could arise from extrinsic changes of environmental constraints (42, 43) and/or in-

trinsic genetic variations, which frequently lead to phenotypic differences, for example, in body size (41, 44). Extrinsic constraints imposed by environments could include temperature, oxygen level, water depth, food availability, predation, and parasitism. It has been demonstrated in modern crustaceans that smaller eggs can better survive high temperature than larger, yolzier eggs (38). Oxygen consumption by small eggs during embryogenesis of extant crustaceans is much lower than that by large ones (45). In ecosystems with high predation risk, the female tends to produce more eggs at the cost of progeny quality (46) since numerous small eggs could offset initial predation during larval dispersal. The egg size of extant crustaceans within an order is typically positively correlated to the body size of the female (44), which might explain the comparatively large eggs of †*W. fieldensis*. It is noteworthy that modern meso- and bathypelagic mysids (Malacostraca: Mysida) show body sizes greater than that of epipelagic species and brood extraordinarily large eggs (\varnothing , 2 to 4 mm) (47). The habitat of †*C. ovata* proved to be a shallow, epeiric shelf (19), whereas †*W. fieldensis* lived in comparatively deep waters (>200 m) at the foot of the Cathedral Escarpment (48). Together, compared with the middle Cambrian †*W. fieldensis*, the early Cambrian †*C. ovata* presumably lived in a habitat with relatively shallower water depth, higher temperature, and higher predation pressure.

Alternatively, the shift in reproductive strategies of the two waptiid species might not have resulted from adaptations to particular habitats, but rather a response to evolution of the Cambrian marine environments through time. The Cambrian period is characterized by the onset of modern-style ecosystems and increasingly complex and diverse interactions among animal species (49, 50). Arthropods developed various adaptations to the ever-changing marine environment in many vital aspects of their biology such as sensing [e.g., compound

eyes and complex brains (51–53), feeding [e.g., sophisticated digestive systems and specialized feeding appendages (53–55)], and locomotion [articulated and diversified locomotory limbs (22, 53, 56)]. Our study suggests that reproduction also took part in the processes of arthropod evolution. We hypothesize the following key evolutionary scenarios in arthropod reproduction: (i) ancestral arthropods released their gametes and underwent external fertilization, as inferred from the trilobite †*Triarthrus* with associated eggs (57); (ii) copulation evolved to increase fertilization success, with females remaining as free spawners and releasing fertilized eggs into the water without brooding; (iii) basic parental care evolved in pioneers such as waptiid and bradoriid arthropods, using their carapace or limbs to carry and incubate the embryos; (iv) some arthropods (including waptiids) underwent scenarios of reproductive trade-offs to optimize the fitness of offspring, as demonstrated by this study; and (v) some stem arthropods further evolved extended parental care to maximize progeny survival and fitness, as implied by the Cambrian fuxianhuidi probably caring for their juveniles (58). Among these scenarios, reproductive trade-offs might have effectively shaped the evolution of early arthropods.

In conclusion, the distinct reproductive strategies in the two closely related Cambrian waptiids might reflect an evolutionary trade-off between quantity and quality of offspring. That trade-off might reflect adaptations to different habitats or, alternatively, an evolutionary response of early pancrustaceans to ecosystem changes through the early-middle Cambrian interval (520 to 508 Ma ago). From an evolutionary perspective, waptiid arthropods might have increased the fitness of their offspring by reaching such a compromise, i.e., decreasing fecundity while at the same time augmenting investment in embryonic provision and parental care. Therefore, the dynamics of balancing the investment between the quality and quantity of progeny might have played a very important role in the success of arthropods during the early stages of their evolution.

MATERIALS AND METHODS

All specimens of †*C. ovata* (16) examined in this study ($n = 1020$) were collected from the early Cambrian Chengjiang Lagerstätte (Yunnan, China) and deposited in the Early Life Evolution Laboratory (ELEM), China University of Geosciences, Beijing ($n = 246$); the Early Life Institute (ELI), Northwest University, Xi'an ($n = 750$); the Yunnan Key Laboratory for Paleontology, Yunnan University (YKLP; $n = 4$); and Research Center of Paleobiology, Yuxi Normal University (YRCP; $n = 20$). Mechanical preparation of specimens preserved with eggs was performed using a Zeiss Stemi 508 stereomicroscope under various light conditions. Three of the four egg-brooding specimens were scanned using a Zeiss Xradia 520 Versa X-ray Microscopes (YKLP) with a scanning energy of 80 kV/7 W and a scanning voxel size ranging from 11.4 to 14.1 μm . Visualization of micro-CT data was rendered using software Drishti 2.4. BSE imaging and EDX analysis of uncoated specimens were conducted with an FEI Quanta 250 FEG field emission scanning electron microscope (SEM) in low vacuum (0.3 mbar) and high accelerating voltage (20 kV). Fluorescence images of eggs were acquired using a ZEISS Axio Zoom.V16 microscope equipped with an FL illuminator (HXP 200 C) under emitted green light (wavelength ≈ 546 nm) at a long exposure time (2000 to 6000 ms) and a mounted camera (AxioCam 503 Color), following the method introduced by Haug *et al.* (59). Specimens were

exposed to blue light; carapace predominantly composed of argillaceous aluminosilicates emitted weak green FL, whereas remains of eggs did not fluoresce. Macrophotographs of fossils were obtained using a Nikon D7000 optical camera under sunlight. Morphological measurements in Table 1 were conducted using Carl Zeiss AxioVision (4.1). Measurements of †*W. fieldensis* were conducted using high-resolution images provided by J.-B. Caron. The diameter of elliptical eggs was measured along the major axes; surface area occupied by eggs was calculated using egg diameter. Bayesian inference analysis of panarthropods was conducted with the addition of †*Chuandianella* to the adult-taxa dataset of Vannier *et al.* (22). Trees were generated using MrBayes 3.2, with two independent runs of 10,000,000 generations and four chains under the Mkv + Γ model; all characters were unordered and equally weighted; trees were sampled with a frequency of every 1000 generations and a burn-in of 25%.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/6/18/eaaz3376/DC1>

[View/request a protocol for this paper from Bio-protocol.](#)

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interests: The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Illustrated specimens of †*C. ovata* are repositied in the Early Life Evolution Laboratory, China University of Geosciences (Beijing); the Early Life Institute, Northwest University (Xi'an); the Yunnan Key Laboratory for Paleontology, Yunnan University; and Research Center of Paleobiology, Yuxi Normal University. Additional and raw data related to this paper may be requested from the authors.

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