

Full length article

Mechanical design principles of avian eggshells for survivability

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ABSTRACT

Biological materials exhibit complex structure-property relationships which are designed by nature's evolution over millions of years. Unlocking the fundamental physical principles behind these relationships is crucial for creating bioinspired materials and structures with advanced functionalities. The eggshell is a remarkable example with a well-designed structure to balance the trade-off as it provides mechanical protection while still being easy for hatching. In this study, we investigate the underlying mechanical design principles of chicken eggshells under various loading conditions through a combination of experiments and simulations. The unique geometry and structure of the eggshell play a critical role in achieving an excellent balance between mechanical toughness and ease of hatching. The effects of eggshell membranes are elucidated to tune the mechanical properties of the eggshell to further enhance this balance. Moreover, a mechanics-based three-index model is proposed based on these design principles, suggesting the optimal eggshell thickness design to improve survivability across a broad range of avian species with varying egg sizes. The survivability-design relationships hold great potential for the development of improved structural materials for applications in sports safety equipment and the packaging industry.

Statement of significance

The fundamental physical principles underlying the complex structure-property relationships in biological materials are uncovered in this study, with a particular focus on chicken eggshells as a prime example. Through the investigation of their mechanical design, we reveal the critical role of eggshell geometry and structure in achieving a balance between toughness and ease of hatching. Specifically, the crack resting effect is observed, making the eggshell easier to break from the inside than from the outside. Additionally, we explore the influence of eggshell membranes on this balance, contributing to the enhancement of the eggshell's mechanical properties. For the first time, we propose a three-index model that uncovers the underlying principles governing the evolution of eggshell thickness. This model suggests optimal thickness designs for diverse avian species, with the goal of enhancing egg survivability. These findings can guide the development of improved structural materials with advanced functionalities, enabling greater safety and efficiency in a wide range of applications.

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1. Introduction

Egg-laying reproduction was one of the most successful evolutionary innovations that enabled vertebrate animals to leave the aquatic environments and colonize the dry land ~360 million years ago [1]. The calcified eggshells produced by all modern birds are considered to have played a critical role in the survival of birds during the Cretaceous–Palaeogene extinction [2]. The significance of the calcified eggshell to embryo development cannot be overstated as it serves three crucial purposes. First of all, it protects the

embryo from the microbial and physical environment. Second, it regulates the water and gas exchange during the incubation. Third, it also provides the calcium necessary for the embryo's skeleton growth [3]. The multifunctions of the eggshell result from its hybrid organic-inorganic composite materials and the sophisticated structures that were developed in millions of years of evolution.

The avian eggshell is a complex bioceramic comprising a calcium-carbonate mineral constituent (~95 % by weight) and an organic matrix (~3.5 % by weight) [4]. The size and thickness of eggshells among various bird species can vary greatly. Furthermore, the shape of the eggshell can differ significantly among species, such as conical in shorebirds, spherical in owls, and elliptical in hummingbirds [5]. In general, the avian eggshell is composed

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of two layers of collagenized fibrous eggshell membranes and multiple calcified layers with different crystal orientations and nanostructures [6]. Some studies have been carried out to explain the structure-function relationship of eggshells. For instance, the varying shape of eggs among different bird species has been linked to their flight strength and efficiency [5]. The organized multi-layered nanostructures of the eggshell undergo changes during incubation, leading to a weakened eggshell and facilitating hatching [7].

Various studies have explored the mechanical properties of eggshells from different bird species and the factors influencing these properties. These include the impact of the loading axis on the rupture of ostrich eggs [8], the influence of egg types on eggshell breaking force [9], and the role of microstructure and crystallographic texture in eggshell strength, which varies with the hen's age. Younger hens, for instance, produce stronger shells due to more uniform crystal orientations [10]. Additionally, research has delved into the mechanics and mechanisms of eggshell failure, examining factors such as fracture toughness and the effect of various defect types and sizes [11]. It has been suggested that microcracks may originate at the contact zone, leading to the propagation of macroscopic cracks [12]. Efforts have also been made to understand the correlation between the eggshell's strength, effective stiffness, and other factors like weight [13,14], calcium carbonate content [15], and egg dimensions [16]. These studies provide valuable insights into the mechanical properties and failure characteristics of eggshells. However, detailed studies on crack formation and propagation using fracture mechanics are lacking. Furthermore, the role of the eggshell membrane in enhancing mechanical properties remains unreported, and there is no well-defined model to elucidate the underlying principles governing eggshell design.

Bioceramics, such as nacre and other exoskeletons with enhanced strength and toughness, serve a straightforward purpose of providing protection for the organisms within [17,18]. However, the design of the eggshell involves a delicate balance of multiple trade-offs. The eggshell must be sufficiently strong to resist various loads applied from the outside in order to protect the embryo, such as attacks from predators, impacts from being dropped or bumped, compressions from being stacked, or forces from the mother adjusting her position. Meanwhile, the eggshell must be easy to break from the inside for ease of hatching. In addition, the eggshell must be tough enough to delay the possible crack growth during the incubation, yet this toughness must be weakened during the time of hatching. Moreover, the eggshell must be thin in order to conserve calcium carbonate, which is limited in birds' natural diets. But it cannot be too thin to resist physical challenges from the environment. Here, we elucidate the underlying mechanical design principles of the eggshell structure using a combination of experimental and computational approaches. Specifically, our study addresses three key questions: (i) Why is the dome-shaped eggshell difficult to break from the outside yet easily breakable from the inside? (ii) What role do the membranes play in affecting the toughness of the eggshell and how to tune the toughness? (iii) How to select the eggshell thickness during the evolution? The fundamental knowledge, in particular survivability-design relationships, holds great potential for the development of improved structural materials, inspired by eggshells, for a wide range of applications in sports safety equipment and the packaging industry.

2. Materials and methods

2.1. Specimen preparation

The chicken eggs used in this study were obtained from a retail outlet and were described as "Large Grade AA Cage-Free Organic eggs." Since potential variations in types [9] and ages [19] of

hens that could affect the material properties and mechanical performance of the eggshell, all eggs used in the tests were obtained from the same source within a short time frame. The external dimensions of the eggshell (a , b , and e) were measured prior to testing and the thickness (t) was measured after testing, as shown in Table S1.

To obtain the compression specimens, the eggs were cut in half using a hand drill equipped with a diamond saw rotating disc. The contents inside the eggs were removed to enable mechanical testing from the inside. Although the contents inside the eggshell can contribute to the mechanical performance of the whole eggs, this would have a minor effect on the breaking force of the eggshell considering the whole egg contains an air cell inside and all mechanical tests are quasi-static [9,11]. The cutting edge was then sanded with 1000-grit sandpaper. The half-eggshell specimens were washed with distilled water and checked under a light source. Specimens with any visible cracks were discarded. Three types of specimens were prepared: specimens with wet membranes attached to them were stored in water; specimens with dry membranes were air-dried for 24 h; for specimens without membranes, the membranes were removed using clamps. The membrane specimens for tensile tests were obtained first by cutting the eggshells in half. Next, the eggshells were rinsed in 1 M HCl to dissolve the CaCO_3 , leaving the organic shell membranes. The shell membranes were then thoroughly washed with water and dogbone-shaped specimens were cut out of the membrane in the latitudinal direction. Half of the specimens were stored in water, while the other half were air-dried for 24 h.

2.2. Mechanical testing

To capture the mechanical response of the eggshell and membrane, compression tests and uniaxial tensile tests were performed using an MTS mechanical tester (C43) equipped with 1 kN and 100 N load cells, respectively. Point loading was used in this study to mimic the actual loading conditions (including contact between eggs, the egg and the surrounding structure, and the egg and the beak) that lead to eggshell breakage in the environment. Note that in a previous study [9], distributed load was also considered, which is not studied here. Both compression tests and tensile tests were conducted in a quasi-static regime with a displacement rate of 0.5 mm/min and a strain rate of 0.001 s^{-1} , respectively. The toughness was defined as the area under the force-displacement curve, representing the energy absorbed and dissipated. This definition is widely used in rigid biological composites. Images of the specimens during the loading procedures were captured at a rate of 1 FPS.

2.3. Numerical analysis

Numerical simulations were conducted using the commercial finite element (FE) package ABAQUS/Standard to capture the stress distribution on the eggshell for various indentation/compression and tensile tests. All the geometric parameters, material properties, and the breaking load applied in the FE model were obtained through previous experimental measurements (see Figures S1 & S2, and Tables S1-S3.). It is important to mention that the eggshell specimens utilized in the tensile test are naturally curved, resulting in a more complex inhomogeneous stress distribution, especially in the throughout thickness direction. The curvature effect is fully considered in our analysis, leading to an elastic modulus of $30.0 \pm 4.6 \text{ GPa}$ (Table S2) and a strength of $19.9 \pm 2.5 \text{ MPa}$ (Table S3) of the eggshell material, which are slightly different from the results in the previous study [9], where the variation throughout the thickness was not considered. More details can be found in

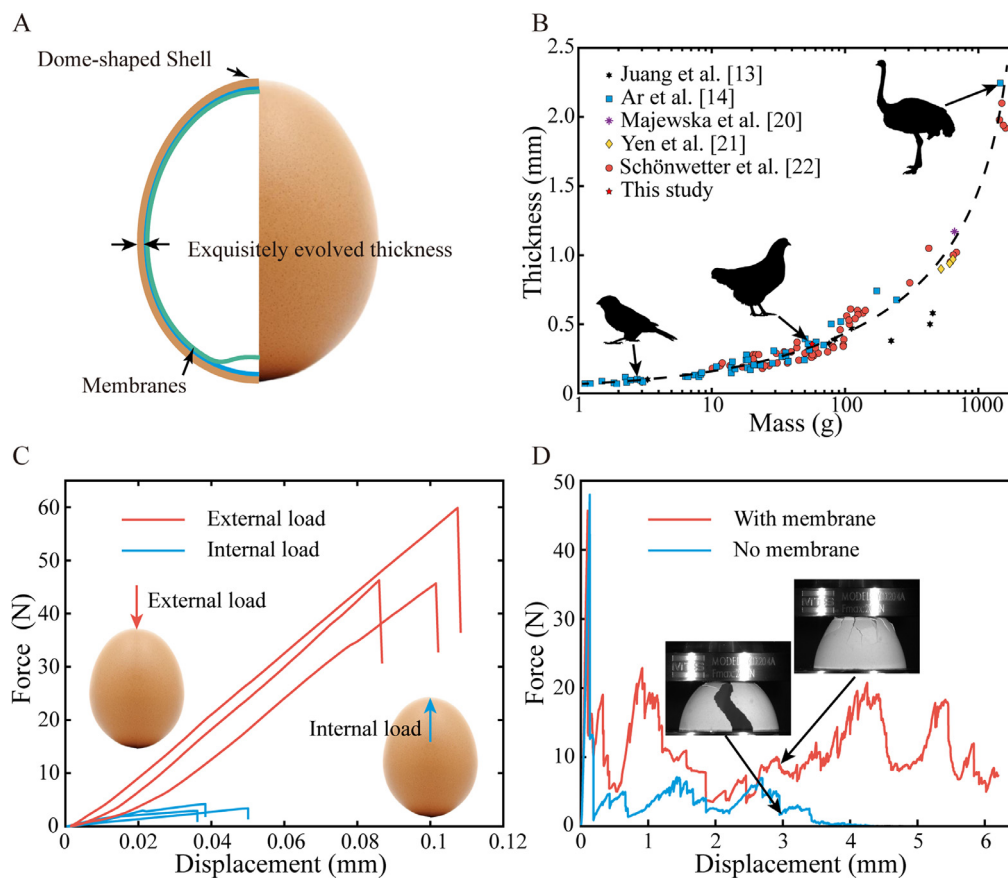


Fig. 1. The structure and mechanical performance of the eggshell. (A) The eggshell structure including the dome-shaped shell, the membranes attached to the eggshell, and the exquisitely evolved thickness. (B) The thickness of eggshells versus the mass of eggs from various bird species [13,14,20–22]. (C) The typical load–displacement curves of the eggshell subjected to internal and external indentation/compression forces, respectively. (D) The representative load–displacement curves from eggshell samples with and without membranes under external compression.

the supplementary information. The model of the eggshell structure is generated using an 8-node linear brick, reduced integration, and hourglass control 3D solid element C3D8R. To ensure accurate stress distributions in the thickness direction, the mesh size in the thickness direction was set to $t/10$ and verified by a mesh sensitivity test. The indenters were modeled with a 3D analytic rigid shell, and contact effects were modeled using a hard contact behavior for the normal direction.

More FE simulations were performed to obtain the stress intensity factors of the cracks. The square root singularity of stress at the crack front region is modeled by shifting the mid-point nodes to the quarter-point locations. This area meshes with collapsed 3D elements C3D15 and the rest of the model is covered with 3D C3D10 elements. The mesh near the crack front is dense to ensure the accuracy of the results. Stress intensity factors were calculated using the ABAQUS built-in J-integral solver based on the domain integral technique. The reliability of the finite element model and the accuracy of the calculated stress intensity were checked by performing convergence tests.

3. Results and discussion

Fig. 1A depicts the typical structure of the dome-shaped eggshell with the membranes and the exquisitely evolved thickness. As the most important geometric parameter, the thickness of eggshells of various bird species, collected from previous studies [14,20,21], is shown in Fig. 1B. It is clear that the eggshell thickness and egg mass have a strong correlation as indicated by the dashed line, depending on the underlying mechanical design

principles of the eggshells. In this study, the eggshells were prepared from large grade AA cage-free chicken eggs. To determine the breaking forces of the eggshell resisting both external and internal loads, two types of indentation/compression tests were conducted on half-shells from inside and outside as illustrated in Figs. 2A and 3A, respectively. The typical load–displacement curves are shown in Fig. 1C, indicating a nearly 20 times larger force needed to break the eggshell from the outside (49.0 N) as compared to the force from the inside (2.6 N). This significant difference can be attributed to different fracture patterns induced by the dome-shaped structure, which will be discussed later in this paper. Similar indentation tests were performed on eggshell samples with the membrane removed to evaluate the effect of membranes on the eggshell's toughness. Fig. 1D shows the representative load–displacement curves from eggshell samples with and without membranes under external compression to a complete failure of the shell structure. Note that the eggshell without the membrane lost almost all of its load-bearing capacity upon fracture. Conversely, the eggshell with the membrane maintained a significant level of load-bearing ability throughout the loading process. The results demonstrate that the membranes play a critical role in enhancing the eggshell's toughness, which we will discuss in detail later.

3.1. Dome-shaped eggshell and fracture patterns

Eggshells are predominantly composed of ~95 % calcium carbonate and can be considered a brittle material. The theory of brittle fracture suggests that, in general, once the maximum princi-

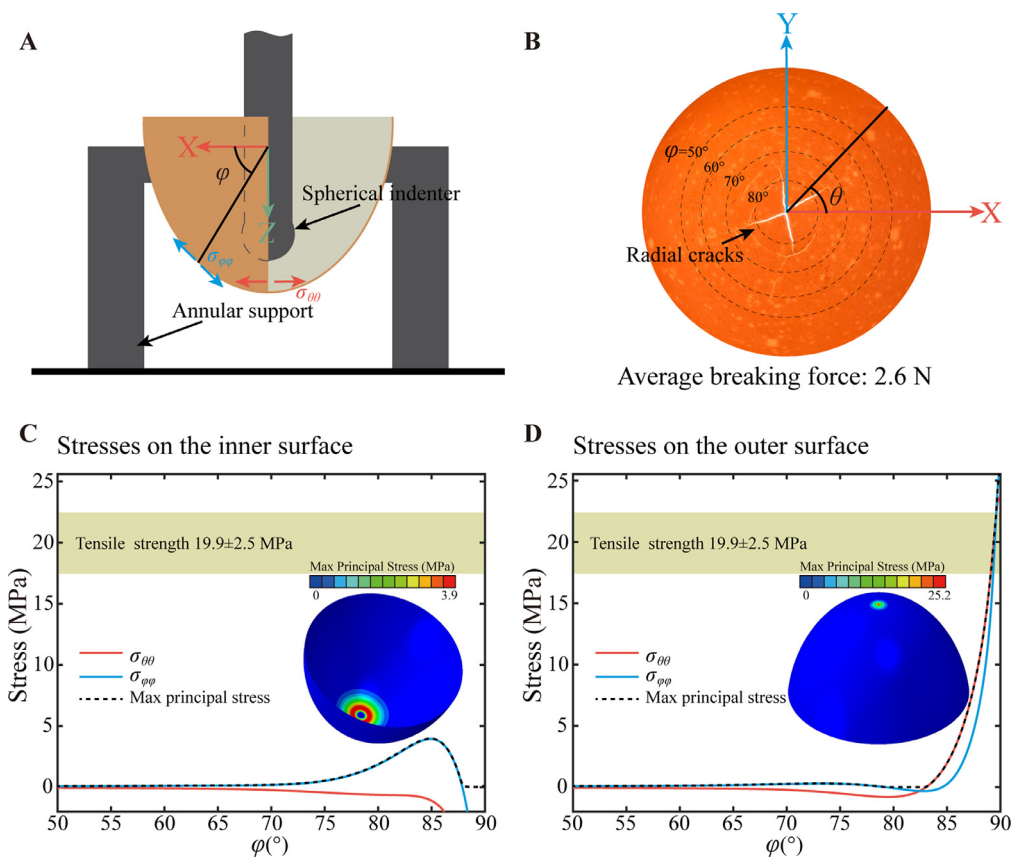


Fig. 2. The mechanical response of the eggshell under indentation from inside. (A) Schematic diagram of the indentation test with an internal load. (B) Four radial cracks initiate at the same location where $\varphi = 90^\circ$ and all propagate radially to $\varphi = \sim 75^\circ$ on the eggshell after breaking. (C) Finite element simulation results of the stress distribution on the inner surface of the eggshell under the breaking load of 2.6 N, where the fracture of the eggshell is not simulated. The maximum principal stress on the inner surface is significantly lower than the tensile strength. (D) Finite element simulation results of the stress distribution on the outer surface of the eggshell under the breaking load of 2.6 N, where the fracture of the eggshell is not simulated. The maximum principal stress on the outer surface reaches the maximum at $\varphi = 90^\circ$, higher than the tensile strength, indicating the initiation of cracks.

pal stress exceeds the tensile strength, small cracks initiate and quickly spread, resulting in a sudden, catastrophic failure. Based on this principle, a hypothesis can be proposed that in the indentation/compression tests on eggshells, cracks will initiate at the point where the maximum principal stress (σ_1) reaches the tensile strength, and will rapidly propagate to cause a catastrophic failure.

Fig. 2B shows the eggshell sample under the internal indentation after the breaking load is reached. Four radial cracks are observed to initiate at $\varphi = 90^\circ$ and advance to $\varphi = \sim 75^\circ$. To investigate how these cracks are initiated, the stress distributions obtained from the finite element simulations are presented in Fig. 2C and D for the inner and outer surfaces respectively. When the breaking internal load (2.6 N) is applied to the eggshell model, the maximum principal stresses on the inner surface are all below 5.0 MPa with the highest value at $\varphi = \sim 85^\circ$. However, the highest maximum principal stress on the outer surface of the eggshell at $\varphi = 90^\circ$ is 25.2 MPa, which is greater than the eggshell's tensile strength of 19.9 MPa (Table S3), indicating all cracks initiate at this point and propagate radially due to high hoop stress component $\sigma_{\theta\theta}$ as expected.

Fig. 3B shows the eggshell sample under external compression after the breaking load is reached. In this case, only one radial crack is observed that initiates from $\varphi = \sim 75^\circ$ and propagates to $\varphi = \sim 55^\circ$. Similarly, the stress distributions on both the inner and outer surfaces are predicted using FE simulation and shown in Fig. 3C and D, respectively. When the eggshell is subjected to the breaking external load of 49.0 N, the highest maximum principal stress is found to occur on the inner surface of the eggshell

at $\varphi = 90^\circ$ as expected. Without simulating the fracture behavior of the eggshell, the highest maximum principal stress is as high as 480.0 MPa, much higher than the tensile strength (19.9 MPa) of the eggshell, indicating crack initiation. However, no apparent cracks are observed experimentally at $\varphi = 90^\circ$ (Fig. 3B). Surprisingly, the crack is found to initiate at $\varphi = \sim 75^\circ$ on the outer surface, which agrees with the simulation results that the highest maximum principal stress on the outer surface is at $\varphi = 79^\circ$, where the predicted maximum principal stress is 17.3 MPa, comparable to the tensile strength (19.9 MPa) of the eggshell. At that location, the maximum principal stress σ_1 is equal to the hoop stress component $\sigma_{\theta\theta}$, which causes a radial crack and is consistent with the experimental observation.

To further explain why the crack initiation on the inner surface of the eggshell under external compression does not cause a failure, the detailed stress distribution on the cross-section region of the eggshell near $\varphi = 90^\circ$ is analyzed using the FE simulation, as depicted in Fig. 4A. The area of the highest maximum principal stress, depicted in gray, is narrow and surrounded by an area of lower stress. If the crack propagates upward, it is likely to quickly reach a region dominated by compressive stress, potentially preventing further propagation. In fracture mechanics, the energetic stability determines whether a crack propagation is 'catastrophic' or 'controlled'. More specifically, if $dK/dC < 0$, the crack is stable, and if $dK/dC > 0$, the crack is unstable and leads to a catastrophic failure, where K is the stress intensity factor and C is the crack length. To better understand the energetic stability of the crack, a 3D FE model was created with a semi-elliptical surface crack as shown

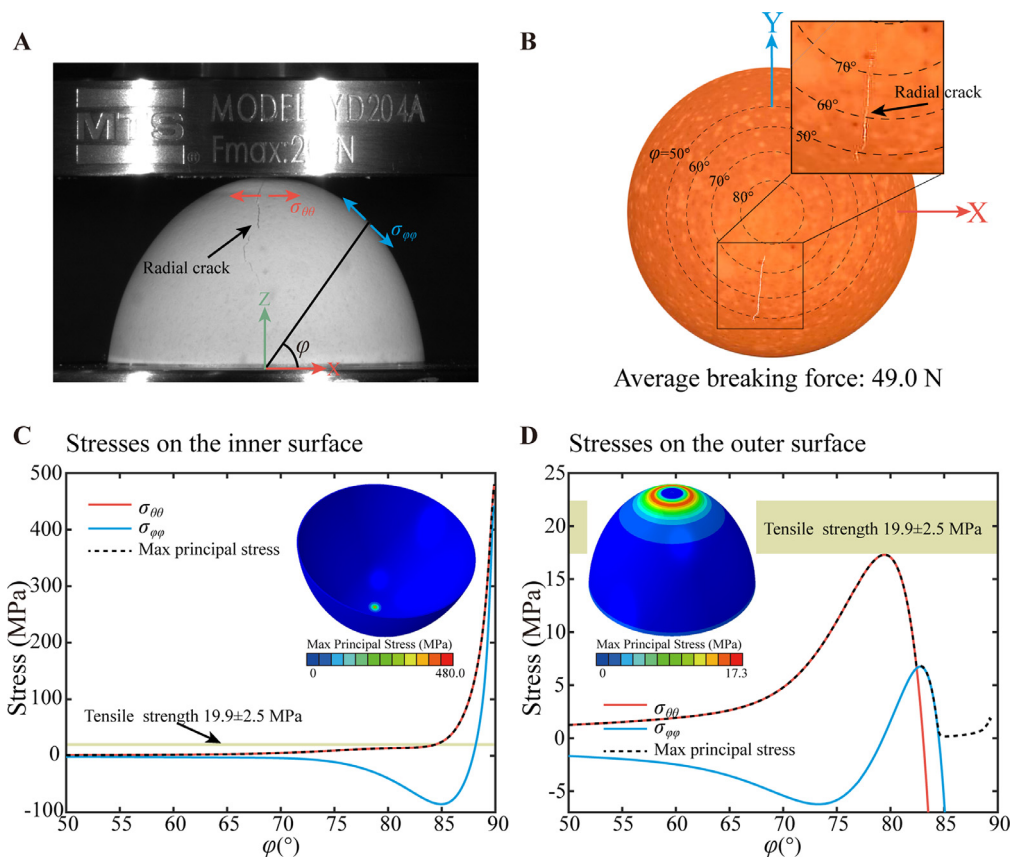


Fig. 3. The mechanical response of the eggshell under compression from outside. (A) Schematic diagram of the compression test with an external load. (B) A radial crack initiates at $\varphi = \sim 75^\circ$ and propagates to $\varphi = \sim 55^\circ$ on the eggshell. (C) Finite element simulation results of the stress distributions on the inner surface of the eggshell under the breaking load of 49.0 N, where the fracture of the eggshell is not simulated. The maximum principal stress at $\varphi = 90^\circ$ is ~ 24 times higher than the tensile strength. (D) Finite element simulation results of the stress distributions on the outer surface of the eggshell under the breaking load of 49.0 N, where the fracture of the eggshell is not simulated. The simulated maximum principal stress at $\varphi = 79^\circ$ reaches the highest value of 17.3 MPa, which is slightly lower than the tensile strength.

in Fig. 4B and D. The stress intensity factors at two crack tips (A and C) are calculated to represent the entire crack front, considering two crack length ratios of $L_a/L_c = 3$ and 6. The calculated stress intensity factors are depicted in Fig. 4E and F for crack tip C and crack tip A, respectively. Note that all four curves demonstrate an inflection point, indicating the change of dK/dC from positive to negative. In the first stage when the crack length is small, $dK/dC > 0$, the crack is unstable and rapidly propagates. After the inflection point of the curve, the crack enters the second stage, $dK/dC < 0$, where the stress intensity factor decreases as the crack length increases. In this stage, the crack propagates slowly and stably until it eventually stops. The maximum lengths of L_a and L_c are 0.25 mm and 1.8 mm respectively where the crack stops propagating. These findings agree well with a previous experimental observation [12], where a micro-crack (invisible to the naked eye) is discovered on the inner surface of the eggshell at $\varphi = 90^\circ$ under a load that was much less than the breaking force, as shown in Fig. 4C. These results reveal a unique two-crack fracture pattern in eggshells under an external load. The initial crack forms on the inner surface at $\varphi = 90^\circ$ as expected, but stops before it penetrates the eggshell. The second crack initiates on the outer surface at $\varphi = 79^\circ$ and propagates rapidly, resulting in catastrophic failure. The resting of the first crack is attributed to the unique stress distribution around the loading point due to the dome-like structure of the eggshell.

Additionally, a validation compression test was performed on the eggshell with geometric imperfections, as depicted in Figure S4. Small holes were introduced at $\varphi = 90^\circ$ (first crack location) and $\varphi = 79^\circ$ (second crack location), respectively, on the eggshell samples. It is found the imperfection at $\varphi = 90^\circ$ does not affect

the breaking force of the eggshell. However, the breaking force for the eggshell samples with a hole at $\varphi = 79^\circ$ is reduced by more than 50 % compared to the average breaking force of 49.0 N. This validation test provides additional support that the failure of the eggshell is attributed to the second crack initiated at $\varphi = 79^\circ$.

Moreover, the loading position on the eggshell under the external load is found to have minor effects on the breaking force as shown in Figure S5. Both experimental and FE simulation results demonstrate that the breaking force is slightly lower when the eggshell is under compression at the equator as compared to the cases of loading at the two ends. This also indicates that the curvature of the dome can play a role in the breaking load. Parametric studies were further performed with different eggshell thicknesses and eggshell curvature as shown in Figure S6. Clearly, the eggshell thickness plays a dominant role and both the internal and external breaking forces increase as the thickness increases. At a given thickness of the shell, it's found the external breaking force is at least 1 order larger than the internal breaking force for all models. More details can be found in the supplementary information.

3.2. Membrane and the toughness of the eggshell

The eggshell membranes are composed of collagenized fibrous tissues that are attached to the inner surface of eggshells. There are two layers of membranes: a thin inner membrane and a thicker outer membrane. The total thickness of these two layers is approximately 0.1 mm [23,24]. The eggshell membrane has been recognized as having three essential functions in avian reproduction. Firstly, it serves as the structural foundation for the calcification

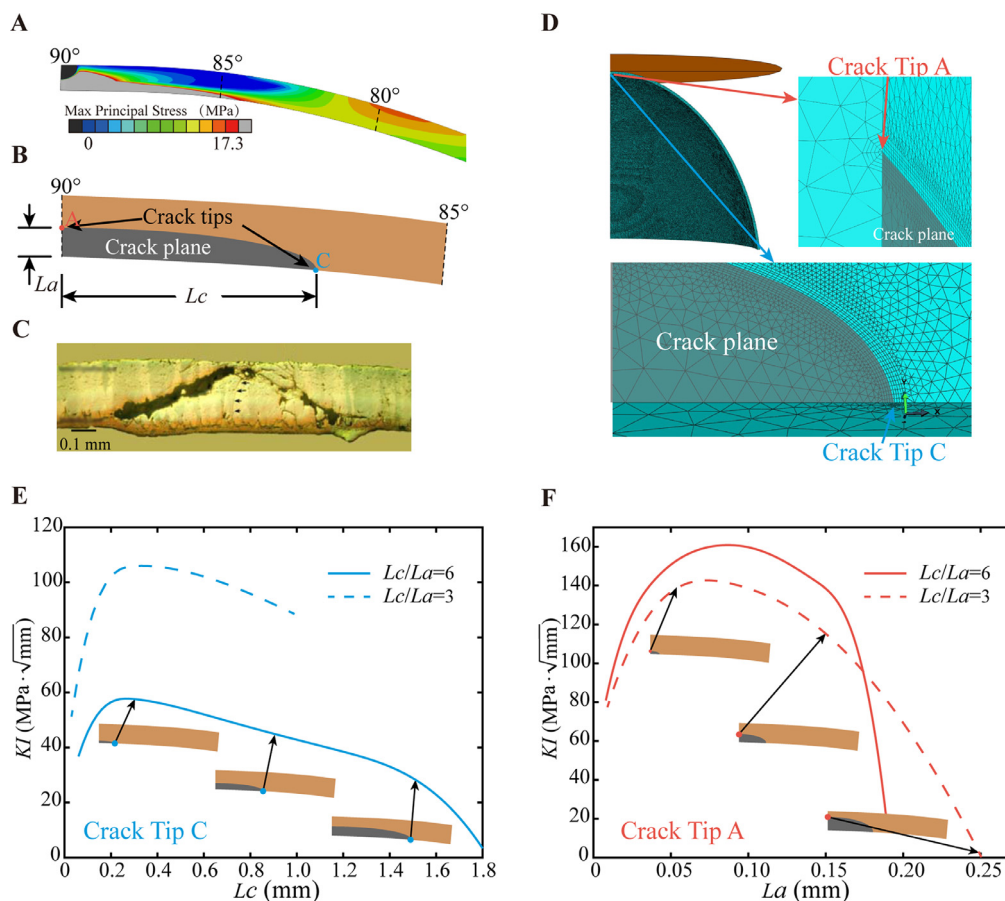


Fig. 4. Crack resting mechanism of the eggshell under external compression. (A) Distribution of the maximum principal stress in the thickness direction near the loading region where $75^\circ < \varphi \leq 90^\circ$. The area with the maximum principal stress larger than 17.3 MPa is shown in gray. (B) Schematic diagram of a semi-elliptical surface crack from the inner surface of the eggshell. The stability of the crack is determined by calculating the stress intensity factors at crack tips A and C. (C) Experimental image of micro-cracks formed near the loading region at an external load less than the breaking load from a previous study [12]. It shows that the first crack does not result in a catastrophic failure. (D) Finite element model and the mesh of a semi-elliptical surface crack on the inner surface. (E-F) The FE simulated results of stress intensity factors at crack tips A and C respectively for a propagating crack. In the second stage, $dK/dC < 0$, indicating that the crack propagates slowly and stably, and eventually comes to a stop.

of the eggshell [25]. Secondly, it regulates gas exchange and facilitates the respiration of the embryo within the eggshell [26]. Thirdly, it acts as a barrier against bacterial penetration during the hatching process [27]. These functions are attributed to the unique structural, chemical, and physical properties of the eggshell membrane, which have garnered attention from numerous researchers across various fields. For example, eggshell membranes have been employed as templates for the fabrication of hierarchically ordered microporous networks composed of oxide materials [28]. They have also been utilized as polysulfide reservoirs in highly reversible Li-S batteries [29]. However, the mechanical properties of eggshell membranes have received little attention. It is shown that the biological soft membrane can impart the natural hydrogel with high strength and fracture toughness, such as in lobster underbelly [30,31]. Therefore, it is expected that the eggshell membrane can play a role in the mechanical properties of the eggshell.

To study the effect of the membranes on the mechanical properties of the eggshell, two additional groups of eggshell samples were prepared. The first group of eggshells had the membranes removed, while the second group retained membranes but air-dried for 24 h before testing. Fig. 5A shows the results of the compression tests on these 3 groups of eggshell samples, indicating no noticeable difference in the breaking forces for all three cases. This suggests that the membrane (wet and dry) has little effect on the strength of the eggshell, given its soft and thin nature. Interestingly, two distinct post-failure behaviors are observed in the force-displacement curves as shown in Fig. 5B. Eggshells without mem-

brane or with a dry membrane lose their load-carrying capacity quickly to below 5 N. In contrary, after the breakage, the eggshells with wet membranes can maintain a high level of force (from 10 N to 20 N) throughout the entire loading process, leading to significantly higher fracture toughness.

The initiation and propagation of cracks are illustrated in Fig. 5C and D for the eggshells with the wet membrane and without the membrane respectively. For the case of eggshell with the wet membrane, the primary radial crack stops midway to the bottom (Fig. 5C). Similar to the organic matrix bridging in nacre [32] and the collagen-fibril bridging in bone [33], the eggshell membrane can also exhibit a bridging effect that distributes the load that would otherwise drive the propagation of the crack. This results in a reduction of the stress intensity factor at the tip of the primary radial crack, thereby halting the propagation of the primary radial crack. However, this also leads to a change in the overall stress distribution and the formation of secondary cracks. After the primary radial crack stops, multiple radial and circumferential secondary cracks alternately initiate and propagate on the eggshell. These two types of cracks are driven by two different types of stress components: one that controls the radial crack ($\sigma_{\theta\theta}$), and another that controls the circumferential crack ($\sigma_{\varphi\varphi}$). These two stress components compete throughout the entire loading process. The presence of the membrane dramatically affects the resulting crack initiation sequence, as shown in Figure S8. For the eggshell with the wet membrane, the first three major drops in the force-displacement curves correspond to three different crack initiations. From points

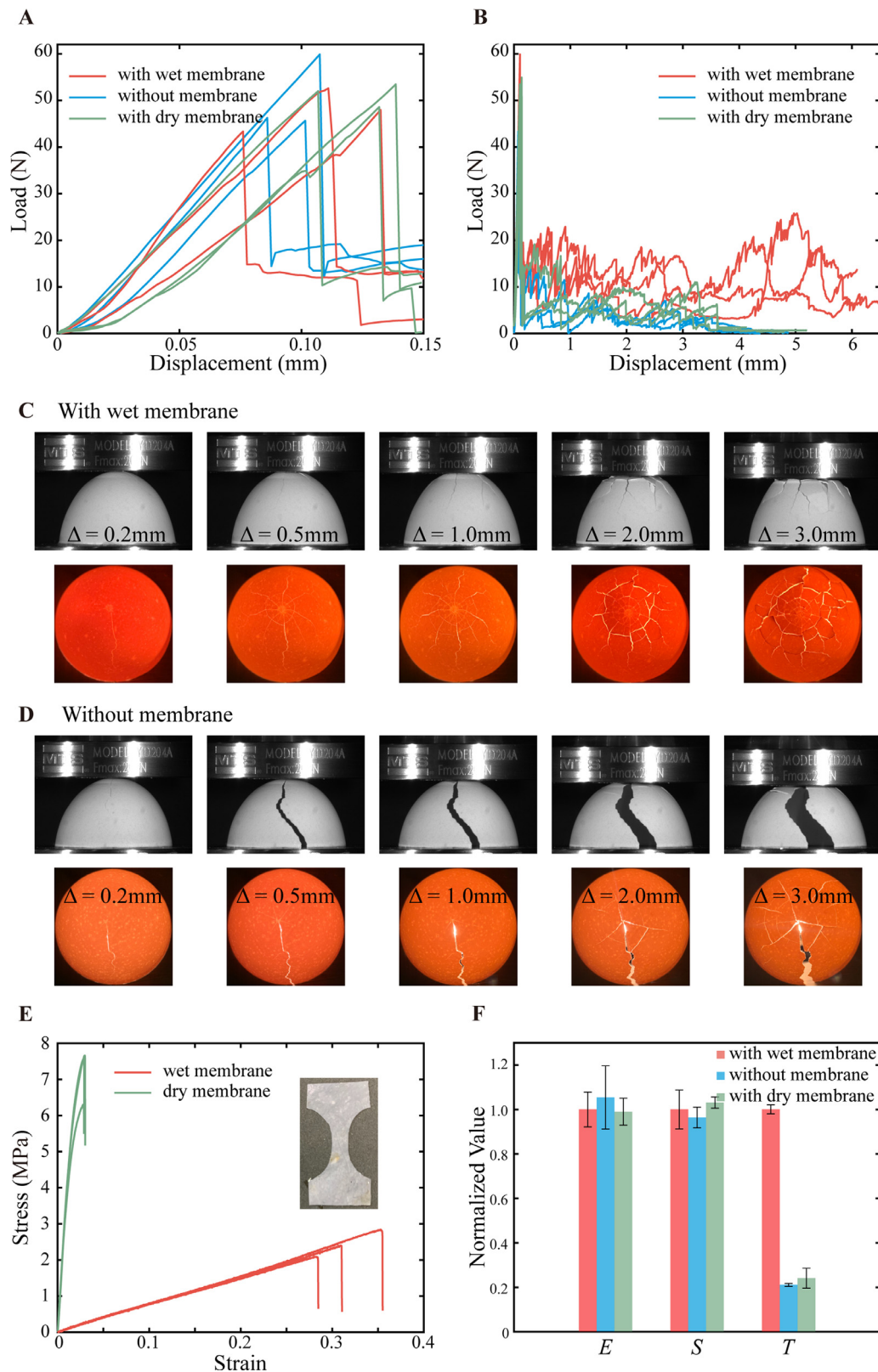


Fig. 5. The effect of the membrane on the mechanical response of the eggshell. (A) Pre-breakage force-displacement curves obtained for three types of eggshells: (1) with membrane attached, (2) with membrane removed, and (3) with dry membrane attached. (B) Post-breakage force-displacement curves of the three types of eggshells. (C) Crack initiation and propagation in the eggshell with the wet membrane attached. Multiple primary radial cracks stop halfway to the bottom. Secondary cracks are generated and then improve the toughness and energy absorption ability of the eggshell. (D) Crack initiation and propagation in the eggshell without membrane attached. A primary radial crack propagates all the way to the bottom, leading to a catastrophic failure. (E) Experimental stress-strain curves obtained for dry and wet eggshell membranes. The mechanical properties of the membrane are significantly influenced by moisture content. The failure strain of the dry membrane is ~ 0.03 , while the failure strain of the wet membrane is 10 times higher to ~ 0.3 . (F) The comparison of three mechanical properties (stiffness (*E*), strength (*S*), and toughness (*T*)) for three types of eggshells: (1) with wet membrane attached, (2) with membrane removed, and (3) with dry membrane attached. The results are normalized by the corresponding values of the eggshell with the wet membrane attached.

1 to 2 and 3, the stress components that win the competition are $\sigma_{\theta\theta}$, $\sigma_{\varphi\varphi}$, and $\sigma_{\theta\varphi}$, respectively, leading to the sequential crack initiations from a radial crack to a circumferential crack, and to another radial crack. In contrast, for the eggshell without the membrane, the primary radial crack propagates all the way to the bottom (Figs. 5D and Figure S7). Due to the differences in stress distribution and crack formation, the eggshell without the membrane has a much lower load-carrying capacity, as predicted by the simulation results.

The wet membrane plays a crucial role in making the eggshell tougher and protecting the embryo inside. However, it also raises a question: does the presence of the membrane impede the hatching process and make it more difficult for the chick to break out of the eggshell? Previous studies have revealed many physical and biological transitions that occur in the eggshell during the incubation process, which facilitate hatching for the chick. For example, the incubated eggshell is 7.3 % thinner than the unincubated eggshell [34], and the pore density of the eggshell increases by about 12 % [35]. During the incubation, avian eggs lose approximately 20 % of water, causing the membranes to dry out accordingly [36]. It has been found that in fertilized chicken eggs, the water content in the shell membranes decreases from 70 % to about 40 % over 17 days of incubation [37]. However, until recently, it was not realized that the change in moisture content of the membrane is also an evolutionary strategy that weakens the eggshell during the hatching process. Fig. 5E shows the comparison of stress-strain curves of the wet membrane and dry membrane. The failure strain of the dry membrane is found to be ~ 0.03 , whereas the failure strain of the wet membrane is 10 times higher to ~ 0.3 . Therefore, when the eggshell with dry membrane breaks, the dry membrane with low failure strain would break as well. But in the wet membrane case, the membrane can remain intact while the crack propagates across the shell. Fig. 5F summarizes the stiffness (E , the slope of the force-displacement curve), the strength (S , the breaking force), and the toughness (T , the area under the static force-displacement curve) of the three groups of eggshells, indicating the wet membrane can significantly increase the toughness of the eggshell. During the incubation period, the moisture content of the membrane decreases, causing the breaking strain of the membrane to decrease correspondingly, leading to a dramatic reduction of the toughness of the eggshell, which in turn assists the chick in breaking out of the eggshell.

3.3. Selection of the eggshell thickness

The mass of eggs and the thickness of eggshells vary across different bird species. For instance, the mass of an ostrich (*Struthio camelus*) egg is approximately 1500 g, with a thickness of around 2 mm [38]. In contrast, the mass and thickness of a chicken (*Gallus domesticus*) egg are approximately 50 g and 0.38 mm, respectively [39].

There have been numerous attempts to elucidate the mechanism of natural selection regarding eggshell thickness. In general, the eggshell must be sufficiently thick to withstand external forces during incubation, yet not so thick as to unnecessarily waste building material or impede the hatchling's ability to break free [14,40]. Many studies have been conducted to uncover the parameters that may affect the eggshell thickness, including egg mass [41], incubator mass [42], and breeder age [43]. These studies have provided regression equations to describe the relationship between these factors and the eggshell thickness. However, while summarizing the phenomenon observed in many experiments, the underlying mechanics are not sufficiently considered.

The thickness of the eggshell appears to be determined by a balance between protection and nutrient consumption, in order to maximize survivability. Reproduction is an energetically costly pro-

cess that requires a sufficient supply of macronutrients and micronutrients. Among these, calcium carbonate is one of the most limiting micronutrients [44]. To quantitatively examine the contradictory relationship between protection and calcium carbonate consumption, we developed a three-index model as shown in Fig. 6A including the production index (PI), the breakage index (BI), and the survival index (SI). The production index, or PI , is defined as:

$$PI(t) = \frac{V_{cc}}{V_{eggshell}(t)}, \quad (1)$$

where t is the thickness of the eggshell; V_{cc} the volume of the available material (calcium carbonate) for eggshell production; and $V_{eggshell}$ is the volume of the eggshell for one egg and it is a function of thickness. The thicker the eggshell, the fewer eggs can be produced using the limited building material. For instance, as shown in Fig. 6A, only 5 eggs can be produced with a larger thickness, t_l , while 20 eggs can be produced with a smaller thickness, t_s . However, producing more eggs does not necessarily result in more surviving offspring numbers because eggs are fragile and may break under environmental loads. The breakage index, or BI , indicates how many eggs break under environmental loads. In the example given, for eggs with a small thickness, $BI(t_s) = 14$, meaning 14 eggs break under environmental loads. The survival index, $SI = PI - BI$, indicating how many eggs survive. Among all three thicknesses, the medium thickness, t_m , has the highest survival index and is thus selected through natural selection.

Since PI can be easily obtained using Eq. (1), determining BI is the key to find the optimal thickness. First, according to our FE simulation, the breaking force F (under external load) can be expressed as a function of the thickness as shown in Fig. 6B. With this relationship, we are able to calculate the probability of egg breaking under different environmental conditions. Here, the maximum environmental load of different eggs is assumed to follow a normal distribution, which is described as:

$$y = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}, \quad (2)$$

where y is the probability density, x is the maximum environmental load, σ and μ can be calculated

$$\sigma = \frac{m \cdot g \cdot k}{x_0 + 3}, \mu = 3\sigma, \quad (3)$$

where m is the mass of one egg, g is the gravitational acceleration, and k is a load factor that is set as 100 here. x_0 can be solved using

$$EF = \frac{1}{\sigma_0\sqrt{2\pi}} \int_{-\infty}^{x_0} e^{-\frac{(t-\mu_0)^2}{2\sigma_0^2}} dt, \quad (4)$$

where $\mu_0 = 0$, $\sigma_0 = 1$. EF is the environmental factor that controls the shape of the probability density function, varying from 0 to 1. A smaller EF indicates harsher environmental condition and a higher probability of large environmental loads acting on the egg. Fig. 6C shows three different probability density functions with EF values of 0.5, 0.7, and 0.9. Then the probability of the eggshell breaking under environmental load can be obtained

$$p = \frac{1}{\sigma\sqrt{2\pi}} \int_F^{\infty} e^{-\frac{(t-\mu)^2}{2\sigma^2}} dt \quad (5)$$

For instance, assume an eggshell has a breaking force of 60 N in a mild environmental condition, $EF = 0.9$, and the breaking probability p is the area of the green region. In a harsh environmental condition, $EF = 0.5$, the breaking probability p is the combined area of the green, blue, and red regions. Finally, the breaking index (BI) is obtained $BI = PI \cdot p$.

Fig. 6D shows the results of the three indexes calculated for eggshells with different thicknesses ranging from 0.2 mm to

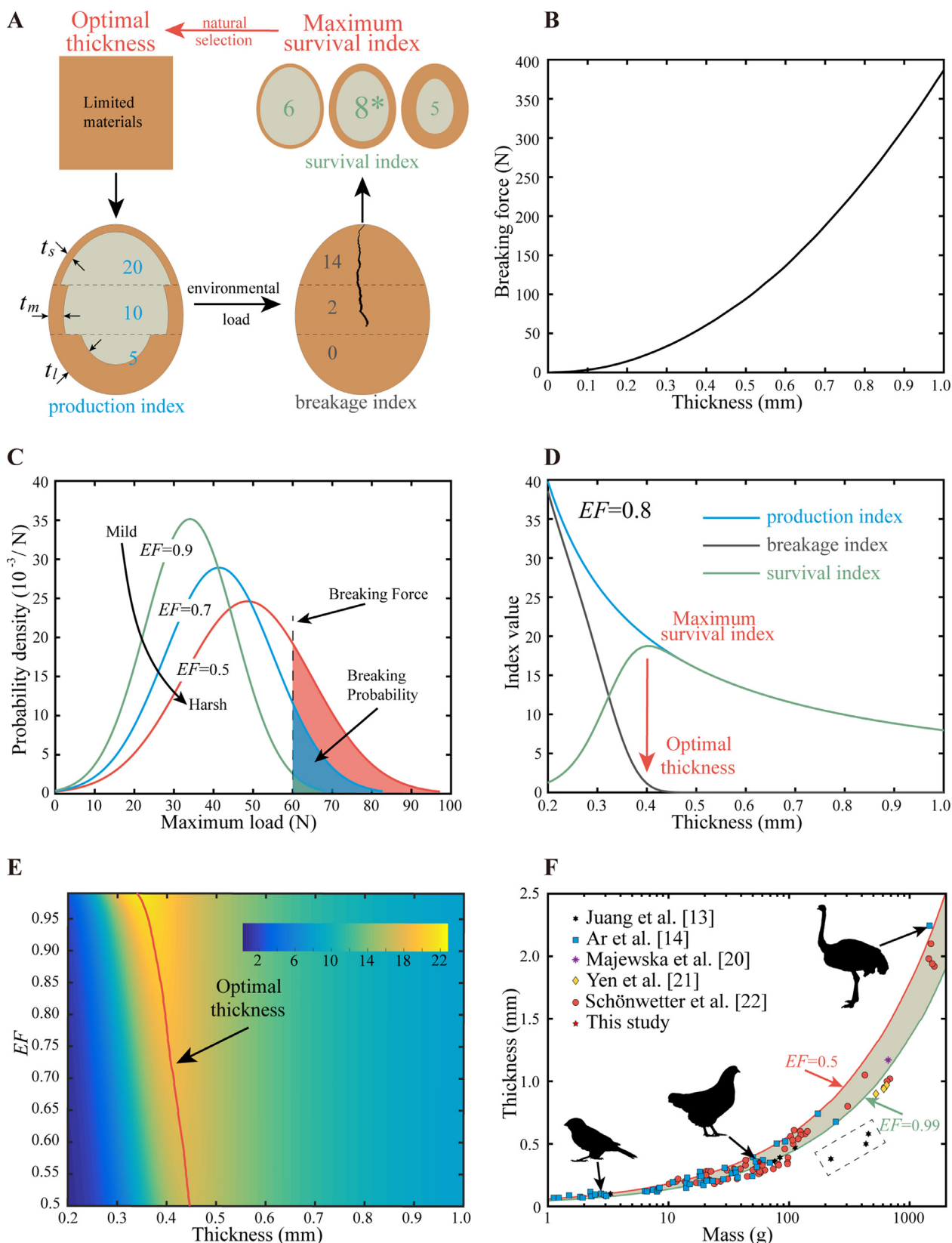


Fig. 6. A possible driving force in the evolution of eggshell thickness. (A) Schematic diagram of the three-index model. The thickness of the eggshell appears to be governed by a balance between protection and nutrient consumption, with the aim of maximizing survivability. (B) The finite element simulation results on the breaking forces of the eggshell as a function of the thickness. (C) Distribution of maximum environmental load of eggshell. EF is the environmental factor that controls the shape of the probability density function. A smaller EF indicates a much harsher environmental condition and the probability of a large environmental load acting on the egg is higher. (D) The production index, breakage index, and survival index for eggshells with different thicknesses. The thickness corresponding to the peak value of the survival index is $t \approx 0.4$ mm which is very close to the average thickness of the eggshell, $t \approx 0.38$ mm, observed from the tests. (E) Survival index of eggshells with different environmental factors and thickness. The optimal thickness is located in a very narrow range, from 0.34 mm to 0.44 mm. (F) Calculated optimal thickness of eggshell for different bird species. Experimental data collected from [13,14,20–22].

1.0 mm when $EF = 0.8$. As the thickness of the eggshell increases, both the production index and the breaking index decrease, but the breaking index decreases at a faster rate. Consequently, the survival index initially increases and then decreases, and the thickness corresponding to the peak value of the survival index is approximately 0.4 mm, which is very close to the average eggshell thickness of $t \approx 0.38$ mm observed. To further validate the hypothetical model, the survival indexes for different environmental factors are calculated and the results are shown in Fig. 6E. The red line indicates the highest survival indexes for different environmental factors. Note that the optimal thickness is ranging within from 0.34 mm (in the mild environmental condition) to 0.44 mm (in the harsh environmental condition).

We further applied this three-index model on other species of birds, where the mass of the eggs ranges from 1 g to 2000 g. Fig. 6F represents the optimal thickness for different environmental factors from 0.5 to 0.99. Evidently, the majority of the data points gathered from previous studies [13,14,20–22] fall within or close to the analytically optimal region. However, three species (*Macrocephalon maleo*, *Apteryx australis*, *Diomedea exulans*) situated in the dashed-board rectangle significantly deviate from the observed trend. This phenomenon was also observed in the previous study [13], where these species possess the longest incubation periods among birds. The three-index model proposed here contains minimal parameters but provides a good range of the optimal thickness and, in general, indicates a trend in eggshell thickness relative to egg weight. The mechanics-based three-index model reveals that the selection of eggshell thickness enables a perfect balance between protection and calcium carbonate consumption to maximize survivability. A more comprehensive model can be developed based on current three-index model by considering additional factors - such as the force required for the chick to hatch, the weight of the bird on the egg during incubation, the size of the clutch, and bacterial intrusion, etc.

4. Conclusion

Avian eggshells have evolved to balance three key design trade-offs for optimal protection against external loads while maintaining easy hatching: (1) The eggshell should be easy to break from the inside, but difficult to break from the outside to protect the developing embryo; (2) The eggshell should be tough during incubation to withstand mechanical loads, but weaker at the time of hatching to facilitate the chick's emergence; (3) The eggshell should be thin enough to conserve building material, yet thick enough to prevent easy breakage. In this study, we have investigated the structure and underlying mechanics of these trade-offs to gain insights into avian development and survival. By examining the crack initiation and propagation of eggshells under different loads, we have discovered two distinct fracture patterns that explain the significant difference in breaking forces between external and internal loads. We have also confirmed the impact of eggshell membrane moisture on the toughness and identified the importance of balancing eggshell thickness to maximize breeding efficiency. Our findings have implications for the development of bio-inspired materials in areas such as sports safety and packaging. Ultimately, a deeper understanding of eggshell mechanics can guide the development of improved structural materials with advanced functionalities, enabling greater safety and efficiency in a wide range of applications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.actbio.2024.02.036](https://doi.org/10.1016/j.actbio.2024.02.036).

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