Micron 101 (2017) 156–161

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Structural characteristics of the core layer and biomimetic model of the ladybug forewing

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ARTICLE INFO

Keywords:
Beetle
Ladybug
Forewing
Microstructure
Trabecular density

ABSTRACT

To explore the characteristics of the core structure of ladybug (*Harmonia axyridis*) forewings, their microstructure was studied using microscopes. The results suggest that trabeculae exist in the frame of the beetle (ladybug) forewing for the first time; this study represents the first determination of the parameters N, the total number of trabeculae in each forewing, and λ_t, the ratio of the cross-sectional area of the trabeculae to the effective area of trabecular distribution. The cross-sectional area of a single trabecula in the ladybug forewing is smaller than those in two other kinds of beetles, *Allomyrina dichotoma* and *Prosopocoilus inclinatus*. However, the average trabecular density of the ladybug forewing is 84 per square millimeter, which is the highest among these three kinds of beetles. The λ_t values are 1.0%, 1.5% and 10.5% for *H. axyridis*, *A. dichotoma* and *P. inclinatus*, respectively, and the corresponding N values are approximately 1.4, 1.7 and 3.7 thousand, respectively. Based on these findings, a biomimetic model of the ladybug forewing is proposed, which is characterized by a core structure with a high-density distribution of thin trabeculae surrounded by a foam-like material.

1. Introduction

Insects, as a form of life that has existed on Earth for 3.5 ~ 4 billion years, currently represent more than 10 million extant species, making them the largest class in the animal kingdom (Matsuka et al., 1992). As the form of life that developed wings earliest among the animal kingdom, insects possess an ability for flight (Sun et al., 2016b, 2017) that plays an important role in their foraging and courtship behaviors, their ability to avoid attacks from enemy species, and the dispersal of their population (Cai et al., 2001). Thus, their wings have become light in weight and exquisitely designed after billions of years of evolution, especially those of Coleoptera. Because the beetle's hard forewings also play a part in protecting its body in addition to their contribution to flying, they usually have excellent mechanical properties, which make them an ideal target for imitation in the development of lightweight, high-strength biomimetic materials (Cai et al., 2001; Thompson, 1961). Abroad, Parker and Lawrence (2001) have studied the hydrophobic-hydrophilic structures of the elytra of the desert beetle *Stenocara* sp. in the Namib Desert. Galusha et al. (2008) have researched the mechanism of the photonic crystal structure found in beetle elytra. Van de Kamp et al. (2016) has mainly studied the shape of the macrofibers, the distribution of the macrofiber layers and the interlocking of the exo- and endocuticles and has compared his own results with those reported previously. At the end of the last century, many Chinese scholars began to study the structures of the elytra of insects of the order Coleoptera (Chen et al., 2015a,b; Sun et al., 2012; Sun and Bhushan, 2012). A series of studies on the surface structures and hardness of beetle forewings as well as their application in biomimetic bulldozing plates were conducted (Cheng et al., 2002; Qaisrani et al., 1993; Ren et al., 1990). And some research on the microstructures and mechanical properties of beetle forewings was also performed (Xiang, 1994; Xiang and Fan, 1994 Xiang and Fan, 1994). Liu et al. (2009) and Yuan (2014) investigated the structural colors of beetle forewings and their applications. The first author of this paper, comprehensively studied the 3D microstructures (Chen et al., 2017a) and mechanical properties (Tuo et al., 2016) of the forewings of *Allomyrina dichotoma* and *Prosopocoilus inclinatus*. This same author also proposed a model of lightweight biomimetic integrated honeycomb plates based on these previous studies (Chen et al., 2015b), and samples of this material were produced (Chen et al., 2016) to investigate their mechanical properties, and then the anti-compressive shared mechanism of the proposed plates was verified (Chen et al., 2017b; Zhang et al., 2017a,b).

In 2016, Xiang et al. (2016) reported the composition, surface morphology and cross-sectional microstructure of ladybug (*Coccinella septempunctata*) wings. Roughly contemporaneously, the microstructure of ladybug (*Harmonia axyridis*) wings was also reported by Sun et al.

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http://dx.doi.org/10.1016/j.micron.2017.07.005
Received 24 May 2017; Received in revised form 15 July 2017; Accepted 15 July 2017
Available online 19 July 2017
0968-4328/

(2015, 2016a). However, neither of these authors mentioned trabeculae. Because most forewing microstructures that had been researched previously were characterized by trabeculae (Chen, 2001; Xiang, 1994), this phenomenon aroused considerable interest in us. Furthermore, upon review, it seems that previous reports concerning ladybugs have mainly concentrated on integrated insect pest control and the ladybug's popularity among insect collectors due to its diet, short growth cycle, colorfulness and beauty. Though there are some studies related to the folding mechanism of hindwing (Saito et al., 2014, 2017), only few studies have investigated the features of ladybug elytra (Cai et al., 2001; Cai et al., 2001; Sun et al., 2012 Sun and Bhushan, 2012). One possible reason for this apparent gap in the literature might be the small body structure of the ladybug, which makes it more difficult to dissect and determine the mechanical properties of the ladybug forewing compared with the forewings of beetles of the Scarabaeoidea superfamily, such as *A. dichotoma*, which are several times larger. The ladybug forewing is soft, not hard as are those of Scarabaeoidea beetles, and its area is small, less than 10% of that of the *A. dichotoma* forewing. In consideration of these features, the structural characteristics of the ladybug forewing might be different. Therefore, the internal microstructure of the ladybug forewing was investigated in this study to serve as the basis for further research on the structural features of the ladybug forewing and the development of new biomimetic applications.

2. Materials and methodology

2.1. Insect collection

The ladybugs (*H. axyridis*) investigated in this study were all collected in Nanjing, Jiangsu Province, China, from May to September of 2016. The sizes of every ladybug and its forewings were measured using a vernier caliper. All beetles were adults measuring 6.7 ~ 7.1 mm in length, 5.3 ~ 5.7 mm in width and 2.5 ~ 2.7 mm in height, with corresponding proportions of approximately 1:2 ~ 2.2:2.5 ~ 3, whereas the forewings were 5.6 ~ 6.5 mm in length, 3.4 ~ 3.8 mm in width and 0.8 ~ 1.13 mm in height. This type of beetle is far smaller than the beetles that the first author of this paper has studied previously, *A. dichotoma* and *P. inclinatus*, which can reach 50 mm in length (Zhang and Li, 2011). Thus, the ladybug studied in this paper can be characterized as a small beetle, whereas *A. dichotoma* and *P. inclinatus* are called large beetles for convenience.

2.2. Preparation of specimens and the instruments used for observation

The ladybugs were dead before observation. The forewings were removed from the ladybugs and dried at room temperature for more than 24 h. Then, cross-sectional specimens were cut from the middle part of the forewings, as shown by the dashed box in Fig. 1(a), and prepared for observation. For specimens that were to be used for observation of the core structure, the lower skins were removed, and some of them were deproteinated by means of boiling in 10% KOH for one hour. The specimens were observed using two kinds of scanning electron microscopes (SEMs), as shown in Fig. 1(b and c). In addition, a metallurgical microscope (XY-MR, Shunyu, China) was also used in this study.

3. Results and discussion

3.1. The structure of the core layer of the ladybug forewing

Cross-sectional images of the ladybug (*H. axyridis*) forewing are presented in Fig. 2(a). For comparison, previously reported cross-sectional images of the ladybug (*C. septempunctata*) forewing (Xiang et al., 2016) and cross-sectional images of the *P. inclinatus* forewing are shown in Fig. 2(b and c). As shown in the sketch at the top of Fig. 2, the cross section of the forewing are characterized by a flat middle region (region II) and edges (regions I and III) that are several times thicker than the middle region. This basic profile is the same as that for large beetles such as *P. inclinatus* (Fig. 2c) and has also been reported previously for the ladybug, *C. septempunctata* (Fig. 2b) (Xiang et al., 2016). Notably, as shown in Fig. 2(a2 and a3), in most cross-sectional ladybug forewing images obtained from three SEM experiments, the core layer appears to be filled with a cavity-containing, mud-like substance, similar to the picture presented in Fig. 2(b2) (Xiang et al., 2016), which is called foam-like material for simplicity (Fig. 2a, stars).

In these three initial experiments on ladybug forewings, only two photographs (Fig. 3a and b) were obtained that suggest the possible existence of trabeculae. However, in the photographs (Fig. 3c-e) acquired from later experiments conducted on forewings with the lower skins removed, the existence of trabeculae in the ladybug forewing was clearly established. Coincidentally, Xiang and Du (2017) also reported the existence of trabeculae in the forewing of another ladybug, *C. septempunctata*, when this paper was under review. Notably, a cracked, clay-like substance could be observed in the core layer around some of the trabeculae or sticking to the upper and lower skins after drying (Fig. 3e). It can be inferred that this substance might exist in the form of a viscous foam material in living beetles, corresponding to the previously observed viscous foamy regions (Fig. 2a2, a3 and b2). And this might be one of the reasons why Xiang et al. didn't observe trabeculae in the report in 2016. However, although the images presented in Fig. 3(a and b) reveal the presence of significant cavity layers around some trabeculae, it is not clear whether obvious voids exist in the core structure according to all photographs acquired in the experiments, as only relatively few photographs of this kind were obtained. Moreover, the transition between the cavity layers and the viscous foam material is not yet clear, nor is their specific distribution. This subject will require further research.

Fig. 1. Sample positioning and instruments used for the observation of the ladybug forewing samples: (a) A outline sketch of the forewings, annotated with the names of the forewing parts; (b) one of the SEMs used for observation (Ultra Plus, Zeiss, Germany); and (c) the other SEM used for observation (Quanta 200 FEI, FEI, USA). In Fig. 1(a), the labels I, II and III denote the three distinct regions of the cross section according to the features of the forewing. Further details are shown in Fig. 2.