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Nipple-like nanostructures covering the corneal surfaces of moths, butterflies, and Drosophila have been studied by electron and atomic force microscopy, and their antireflective properties have been described. In contrast, corneal nanostructures of the majority of other insect orders have either been unexamined or examined by methods that did not allow precise morphological characterization. Here we provide a comprehensive analysis of corneal surfaces in 23 insect orders, revealing a rich diversity of insect corneal nanocoatings. These nanocoatings are categorized into four major morphological patterns and various transitions between them, many, to our knowledge, never described before. Remarkably, this unexpectedly diverse range of the corneal nanostructures replicates the complete set of Turing patterns, thus likely being a result of processes similar to those modeled by Alan Turing in his famous reaction–diffusion system. These findings reveal a beautiful diversity of insect corneal nanostructures and shed light on their molecular origin and evolutionary diversification. They may also be the first-ever biological example of Turing nanopatterns.

Biological patterning at the microscale and macroscale levels has been under intensive investigation by developmental biology, and its fundamental principles, such as the concept of the morphogens, have become textbook knowledge (1). In contrast, nanoscale biological patterning is not well studied and understood. Among the rare known examples of biological nanopatterns are the 3D nanocoatings covering insect corneal surfaces (2). They were described in moths and butterflies and later some Dipterans as pseudoregularly spaced nipple-type protrusions, up to 200 nm in height and width (3–7). These nanocoatings may carry antireflective, dirt-removing/self-cleaning, and hydrophobic/antiwetting functions (2, 8–12). Later, some other insects were found to possess a very different type of corneal nanocoating, such as the antireflective maze-like 30-nm-high evaginations covering corneae of the overwater eyes of Gyринidae beetles (13). An attempt to analyze the variety of corneal nanocoatings throughout the insect class was made in the classical study by Bernhard et al. (5). However, the scanning electron microscopy technique of that time was mostly performed on platinum replicas of the insect samples and was compromised by the partial collapse of the nanoprotrusions. It permitted reliable identification of 50- to 250-nm-high nipple-type protrusions in Lepidoptera, some Dipterans, Trichopterans, and, interestingly, the primitive Thysanurans, but not identification of other types of corneal nanocoatings (5).

To use the corneal nanocoatings as the model to study nanoscale biological patterning, a comprehensive investigation across insect lineages using modern techniques must be performed. We recently applied atomic force microscopy (AFM), providing nanometer and subnanometer resolution of undamaged biological material, to investigate different types of corneal nanostructures of some Dipteran and Coleopteran insects (6, 13).

Here we expand this analysis to 23 insect orders and some noninsect arthropods, describing a striking richness and beauty of the corneal nanocoatings (Fig. 1, Figs. S1–S3, Table S1, and Detailed Description of Diverse Corneal Nanocoatings Order by Order). These nanocoatings can be grouped as follows. (i) Nipple-like structures (Fig. L4 and Fig. S1) include the regularly packed protrusions of Lepidopterans (Fig. SL4), irregular packaging in Dipterans (Fig. SL5), and irregular packaging of irregularly shaped nipple-like protrusions in a range of other orders: Trichoptera (Fig. L4), Mecoptera (Fig. SL6), Megaloptera (Fig. SL1D), Hemiptera (Fig. S1E and F), Psocoptera (Fig. S1G), Thysanura (Fig. SLH), Raphidioptera (Fig. SLJ), Neuroptera (Fig. SLJ), Orthoptera (Fig. S1K), and Odonata (Fig. SLL). (ii) Maze-like nanocoatings (Fig. L8 and Fig. S2) can be observed in Coleopterans (Fig. S2A and B) but also in other orders such as Trichoptera (Fig. L8) and Hymenoptera (Fig. S2C), and in some arachnids (Fig. S2D and E). (iii) Parallel strands/ridges (Fig. LC) formed by fusion of nipple-type protrusions can mostly be seen in Dipterans (Fig. L F and G) and, interestingly, in true spiders (Fig. LC). (iv) Novel dimple-type nanocoating (Fig. LD and Fig. S3) can be seen in different orders: Siphonaptera (Fig. S3A), Coleoptera (Fig. S3B), Hymenoptera (Fig. S3C), Hemiptera (Fig. S3D and E), Blattodea (Fig. S3F), and Dermaptera (Fig. LD), and, interestingly, in centipedes (Fig. S3H). We also see various transitions between these major forms: (i) nipples-to-maze transition (e.g., in

Significance

Corneal surfaces of some insects are coated with nipple-like nanostructures reducing the light reflection. Here we provide an extensive analysis of corneae across insect groups. Using atomic force microscopy, we discover a striking diversity of corneal nanocoatings, omnipresent in arthropods. These fascinating bionanostructures replicate the complete set of the Turing patterns—shapes resulting from the reaction–diffusion modeling underlying many examples of patterning in biological and physicochemical systems. Our work, verging on the interface of nanotechnology and zoology, evolution and biophysics, and ecology and genetics, sheds light on the molecular origin and evolutionary diversification of a beautiful diversity of insect corneal nanostructures. It also describes, to our knowledge, the first-ever biological example of Turing nanopatterns.
Plecoptera, Fig. 1E); (vi) maze-to-strands transition (e.g., in Diptera, Fig. 1F); (vii) nipples-to-strands transition (e.g., in Diptera, Fig. 1G); and (viii) dimples-to-maze transition (e.g., in Hymenoptera, Fig. 1H).

The rich diversity of these nanostructures and the easiness with which the corneal nanopatterns merge one into another in closely related orders and even within the same family (Fig. 2 and Detailed Description of Diverse Corneal Nanostructures Order by Order) is striking and permits posing questions on the underlying molecular, developmental, and evolutionary mechanisms. Developmentally, the nipple-type protrusions were proposed to originate, during eye development, from secretion by the regularly spaced microvilli of the cone cells (5, 14). However, this idea could appear plausible when the ordered Lepidopteran nipple arrays were studied but, with the current diversity of nanostructures and transitions among them, sometimes within the same lens (Fig. 1G), is not satisfactory. Instead, we propose that certain mechanisms of patterning at the nanoscale are in place, and the diverse arthropod corneal nanostructures we describe here represent a model to study such nanopatterning. Further, we notice that this diversity of corneal nanostructures is remarkably similar to the complete set of the Turing patterns (Fig. 3).

In his seminal paper in 1952, Alan Turing provided a system of differential equations describing the reaction–diffusion system of two reacting morphogens—a slowly diffusing activator and a fast diffusing inhibitor—which can model various biological, chemical, and physical patterns (15, 16). Applicability of this model to biological pattern formation has been shown in several recent examples, such as formation of colored stripes in zebrafish (17), hair follicle spacing in mice (18), and digit specification in limbs (19). The insect corneal nanopatterns we describe here differ from these examples, as they reproduce not just one of the many possible forms produced by the reaction–diffusion model but a thorough set of possible variants including the intermediate forms (Figs. 1 and 3). This remarkable completeness of coverage of the possible set of Turing structures by the arthropod corneal nanopatterns strongly argues in favor of the hypothesis that these nanopatterns are indeed a consequence of the Turing reaction–diffusion mechanisms.

We hypothesize that the Turing mechanism-based reaction–diffusion processes patterning the nanocoatings are mediated by organic components of the lens, possessing different diffusion properties and mutually influencing each other’s abundance/polymerization/aggregation, the outcome of this being the stereotypical formation of the nanostructures. In previous applications of the Turing principles to biological processes, patterning at the microscale was modeled (17–19). Formal mathematical analysis shows how key parameters of the reaction–diffusion equations (primarily the diffusion coefficients of the two interacting morphogens) can result in the appearance of repeated developmental structures with the experimentally observed micrometer-scale wavelength (20). Our mathematical analysis (Turing modeling of corneal nanopatterns) demonstrates that nanoscale patterns are expected to form in the reaction–diffusion system acting in the colloidal or liquid crystal-type environment [which is indeed the environment of the lens of the eye (21)] where diffusion properties are reduced (compared with the liquid phase).

Although the molecular identity of the morphogens patterning corneal nanocoatings remains to be revealed, simulations of the Turing reaction–diffusion processes provide interesting hints into the potential molecular mechanisms underlying formation of different types of the nanocoatings and transitions among them (Fig. 3I and Fig. S4). Although different sets of the reaction–diffusion coefficients (like that of Table S2 used to obtain images on Fig. 3 and Fig. S4; see also Fig. S4B for schematic description of the parameters and Fig. S5 for analysis of the parameter space) can model different nanopatterns, simulations find that three of the major types of patterns we observe in insect corneae occupy defined regions within the parameter space and transit to each other as follows: dimples ↔ maze ↔ nipples (Fig. 3J and Fig. S4A). The space in these figures is populated by the incremental changes of
two of the reaction–diffusion parameters $a_v$ and $b_u$ describing the degree of influence of the two diffusing components (activator $u$ and inhibitor $v$) on each other (Fig. S4B) (16, 22). Interestingly, transition from the dimple-type nanocoating to the maze-type and then further to the nipples occurs by increasing the absolute value of either of the two reaction–diffusion parameters (Fig. 3I and Fig. S4A). In this regard, it may be speculated that the initial reaction–diffusion nanopatterning system emerged when these parameters just exceeded the borderline, permitting the Turing patterns to appear (16, 22), and thus was likely of the dimple type. In this regard, it is interesting to note that the dimple pattern is not only seen in many insect groups but also in centipedes (Fig. S3H), which are believed to retain more characteristics of the presumed arthropod ancestor than other arthropods with sequenced genomes (23).

In the context of phylogeny, evolutionary advanced insects were initially assumed to possess fully developed nipple-type corneal nanocoatings, whereas simpler insects were reported to mostly carry less pronounced (and less functional) nanocoatings (5). Our findings suggest that this assumption is incorrect. Indeed, our study unequivocally shows that various types of the nanostructures can be seen in various insect (and wider—Arthropodan) groups without any correlation of the predominant type of the nanocoating and the evolutionary advance of the group (Fig. 2). Instead, we can also apply the Turing modeling to get insights into the evolutionary transitions among different types of corneal nanocoatings. Increase in the absolute value of either of the two $a_v$ and $b_u$ parameters allowed the dimple-to-maze transition, and the further increase allowed the maze-to-nipples transition (Fig. 3I and Fig. S4A). These considerations permit constructing a “morphogenetic tree” or “morphogramme”

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Fig. 2. Distribution of basic and intermediate nanopatterns among insect orders. Each pattern type is represented by a circle of a certain color on the diagram; double-colored circles correspond to transitional nanopatterns. Orders of which no representatives were analyzed in the present study are marked as N/A. The data on insect phylogenetic relationship are based upon ref. 24.
of these structures (Fig. 4). In this morphogramme, different types of corneal nanocoatings are placed not based on the phylogenetic hierarchy of the insect orders (24) but instead on their morphologies and transitions among them, as justified by the Turing modeling we performed. Originating from the dimple-type nanocoatings, this morphogramme then grows into the maze type and further into the nipples type (Fig. 4). We further identify parallel ridges of some Dipterans and hexagonally packed nipples of some Lepidopterans as developments of the maze- and nipples-type structures, respectively (Fig. 4). Both represent more ordered structures and can be modeled to emerge from their less ordered predecessors through increasing of the diffusion parameter of the activator component $D_u$ to the levels maximally allowed within the boundaries permitting the Turing patterns to form (16, 22) (Fig. S4 C and D). In contrast, increasing the diffusion parameter of the inhibitor component $D_v$ leads to increase in the cross section of the nanostructures (nipples or ridges, respectively, in Fig. S4 C and D).

In some Dipterans, a transition from nipples to parallel ridges can be seen within the same lens, with nipples occupying the central part of the ommatidium and merging into elongated strands away from the center (Fig. 1G). Turing modeling predicts how such structures may be formed (Fig. S4E). Seen in flies with large ommatidia and lenses, these nanocoatings are likely a result of a nipples-to-maze transition within the same lens, happening during the lens formation after initial nipples in the center of the cornea have been formed. In this predefined space, parameters otherwise giving rise to mazes induce formation of parallel ridges emanating from the nippled area (Fig. S4E).

**Fig. 3.** The insect corneal nanostructural diversity replicates Turing patterns. Mathematically modeled Turing patterns (in black and white) and their insect counterparts. (A and A') Irregular nipples of various sizes, characteristic e.g., for Hemipteran corneal nanocoatings. (B and B') Highly ordered nipple nanoarrays (Lepidoptera). (C and C') Strands merging into a maze (Diptera, Tabanidae). (D and D') Parallel strands (Diptera, Tipuliidae). (E and E') Nipples merging into a maze (Plecoptera). (F and F') Typical maze-like structures (Coleoptera, Gyrinidae). (G and G') Angular maze-like structures (Coleoptera, Coccinellidae). (H and H') A typical dimpled pattern (Dermoptera). A' is a fragment of Fig. S1F; B' is an image from a Pterophoridae butterfly; C' is a fragment of Fig. 1E; F' is an image from a Gyrinus beetle [overwater eye (13)]; G' is a fragment of Fig. S2B; and H' is a fragment of Fig. 1D. Modeling parameters are given in Table S2. (I) Simulations of Turing patterns formation. Step-wise changes in the $a_v$ and $b_u$ parameters within the boundary conditions produce different Turing patterns: dimples (yellow zone), mazes (blue zone), and nipples (green zone). See Fig. S4A for more detailed representation.
Detailed analysis of the physical (such as antireflective and antiwetting) properties of the diverse corneal nanostructures we present here is still to be performed, but the fact that both the nipple-type and maze-type nanostructures serve the antireflective function (2, 13) suggests the functionality of the majority, if not all, of them. The variety of these nanostructures can serve as a highly promising model, obeying the Turing mechanism of pattern formation. Insect eyes, especially those of the genetically tractable model insect Drosophila melanogaster (6, 25), can therefore serve as a powerful tool to further explore the precise mechanisms of the reaction–diffusion-driven processes in living organisms, to identify the molecular components governing formation of corneal nanocoatings, and to genetically engineer novel Turing nanopatterns with novel physical properties.

Methods

Insect Specimens. The dried insect samples were obtained from a collection of the Department of Entomology, Moscow State University. Fresh specimens were collected in the woods around the town of Pushchino, Moscow region. The phylogenetic tree of the insect class was taken from Su and coworkers (24).

Atomic Force Microscopy. To prepare corneal samples, the head of an insect was cut out of the body, followed by removal of the mouth apparatus with a scalpel, splitting of the head into the two hemispheres, and careful extraction of the brain tissue with forceps. Next, the cornea was cleared from the head capsule tissue as well as the underlying brain material with a scalpel. The sample was attached to a glass slide for AFM by means of two-sided scotch tape. AFM scanning of the corneal surfaces was performed with the Integra-Vita microscope (NT-MDT). For the semicontact procedure, the nitride silicon cantilever NSG 03 (NT-MDT) was used. The parameters of the cantilever were: length, 100 μm; resonant frequency, 62–123 kHz; radius, 10 nm; and force constant, 0.4–2.7 N/m. For the contact procedure, the cantilever CSG 10 (NT-MDT) was used, with the following parameters: length, 250 μm; resonant frequency, 14–28 kHz; radius, 10 nm; and force constant, 0.03–0.2 N/m. The choice between the semicontact and the contact measuring procedures was dictated by the size and curvature of the studied surface of the sample but provided essentially identical results. In each AFM experiment, several scans were made to check the reproducibility of images and the absence of possible surface damages. Measurements of height and width of the corneal nanostructures were performed by the Nova software (NT-MDT).

Turing Modeling. The 2D patterns were made using the software RDsim1.jar (16) with the parameter values listed in Table S2.

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Fig. 4. Transformations of corneal nanopatterns. The morphogramme depicts the likely interconversions among the nanostructural patterns found in the insect class rather than phylogenetic relationships of the patterns. Primordial dimpled nanopatterns (1, here from a Farficula earwig) can transform into various maze-type nanostructures (2–4; 2 from a Pyrrhocoris firebug, 3 from a Tabanidae fly, and 4 from the butterfly Protographium asius). The latter can further transform into disordered nipples (6, here from the fruit fly Drosophila melanogaster), which can further become orderly packed (7, here from a Pterophoridae moth). Alternatively, parallel ridges (5, here from a Tipulidae fly) can evolve either from mazes or nipples. The figure is made of reconstructed 3D AFM images fused, for the sake of visualization not in exact scale, using MATLAB.