Title: Changing rounds into squares or combining stripes: Diversity and formation of checkerboard patterns in Eukaryotes

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Abstract

Important in many human artistic cultures, checkerboard patterns are rare in nature like many motifs based on squared geometry. Nevertheless, they are expected to be very detectable by the visual networks due to their periodic geometry, and diverse plant and animal species bear them, suggesting specific biological functions. Here, thanks to a biological survey, we first draw the diversity of species bearing checkerboard patterns. Then, we selected two genera, **Sarcophaga** ***flies and*** **Fritillaria** ***flowers to perform simulations and functional studies to decipher the mechanisms producing these very peculiar patterns. Although they share a similar geometry, these two genera appear to produce checkered patterns through two very different ways, showing a convergence of shape but not of mechanism. Together, this shows the extent of the mechanisms selected during evolution to generate complex forms, and confirms the importance of describing color patterns through the species diversity***

Introduction

Contrary to hexagons and circles, squares patterns are much more uncommon in nature and many studies have proven the difficulty to produce squares and the instability of such patterns[1 2]. Nevertheless, square lattices (to which checkerboard patterns belong) have been described in some eukaryotic tissues, like the mouse auditory epithelium[3] or the oviduct epithelium of the Japanese quail[4]. Concerning color patterning, checkerboard patterns are the combination of two characteristics: a square lattice, and an alternation of coloration. The latter is very common in color patterning, and many processes are able to produce them, like the ubiquitous Turing-like mechanism[5]. Two possibilities coexist, either squares and color alternation are directly produced by the same processus, (in the same way that in a Turing-like pattern the dots and their hexagonal arrangement are produced by the same mechanism[6]), or produced by two separated mechanisms, simultaneously or not, which combine. As they possess a double periodicity, they are expected to be easily detected by visual systems[7 8 9].

If some species are quite famously known for their checkerboard color patterns like the *Fritillaria* flower (Liliaceae) which bear them on their tepals[10], few species have been formerly described for those peculiar shapes, and no work has already presented them combined in a single study. We then chose to describe as many checkerboard patterns as possible, using multiple data sources, taking care to cover the maximum of eukaryotic biodiversity.

After the species survey, a large range of potential species to study was available and we decided to choose and compare an animal and an angiosperm species (and their genera): *Sarcophaga* flies and *Fritillaria* flowers. They presented the patterns closest to a perfect checkerboard motif, and were accessible to functional studies, although not genetically. Simulations, microscopy observations and species comparison allowed us to propose two distinct mechanisms of formations, thanks to a cluster of clues.

Results

Checkerboard color patterns are rare but present in many diverse taxa of Eukaryotes, notably plant and animal species

To perform a survey of species bearing checkerboard patterns, we first had to define them properly. We chose to consider two separate characteristics of checkerboard patterns: square shaped-motifs and an alternation of two colors [11]. Perfect checkerboard patterns are never reached by real patterns, so we chose to extend the definition and include species with slightly diverging characteristics, like not perfect squares or partly irregular color alternation. Since no exhaustive survey was possible, we chose to use keywords to find the species (‘checkerboard’, ‘checkered’, ‘square’ and ‘*fritillaria’*, the latin word for ‘dice cornet’, a object exhibiting a checkerboard pattern and then used in species names to pinpoint the presence of this particular pattern, or one close to it[12]).

Thanks to this, we described several species and genera, summarized in Figure 1, and qualitatively represented in a diagram thanks to the more or less perfectness of their characteristics. Both angiosperms and animals were found to exhibit checkerboard-like patterns, but we were not able to find any species belonging to Fungi. Very diverse animal species (vertebrates, insects, echinoderms, cephalopods) were found, but few plant species seem to bear such patterns. The two most perfect checkerboard-like patterns were found in *Sarcophaga* flies genus, exhibited on their abdomens, and *Fritillaria* genus, with *Fritillaria meleagris*. We then chose these two groups to explore pattern formation.

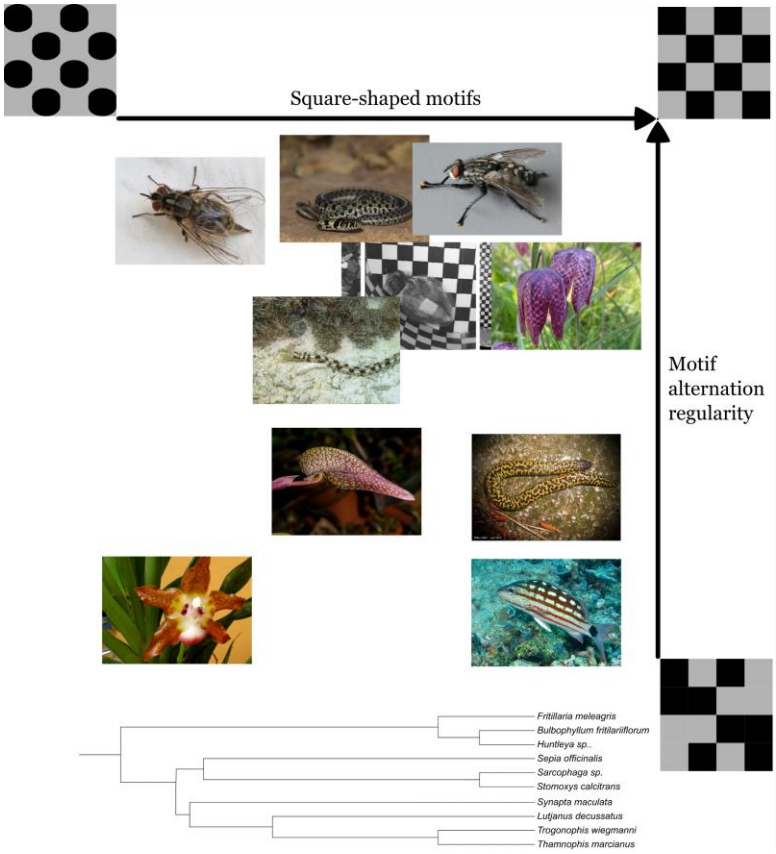

Figure 1. Checkerboard-like patterns of Eukaryotes species. x-axis: distance to perfect squares motifs. y-axis: pattern alternation regularity. From up to down and left to right: Stomoxys calcitrans, Thamnophix marcianus, Sarcophaga sp., Synapta maculata, Sepia officinalis, Fritillaria sp., Bulbophyllum fritilariiflorum, Trogonophis wiegmanni, Huntleya sp., Lutjanus decussatus.

Fritillaria *checkerboard patterns are compatible with a Turing-like mechanism constrained by a parallel nervure frame*

To extract clues of *Fritillaria* mechanisms producing their checkerboard-like patterns, we performed microscopy on fresh individuals (Figure 2.A and B) and also from museum specimens presenting interesting features on their tepals, such as slight deviation from the classical patterns. We found that depending on the species, colored shaped go from squares (notably in *F. meleagris*) to sponge shapes for the pigmented areas (i.e. straight on the sides touching the nervures and with a negative curvature on the other two sides), and round shapes for the unpigmented areas. In the most distal part of the tepals, sponge colored shapes tend to break into two parts (Figure 1.B, left panels). In some individuals, especially

in the *Fritillaria meleagris* museum specimens, we observed a very interesting feature in the places where the nervures are the widest: instead of alternations, several motifs were side by side, suggesting that nervures act as constraining barriers, but when the patterning mechanisms has sufficiently space, a spot pattern is produced. Given the periodicity of this pattern, and the round geometry of the motifs, this strongly suggests a Turing-like mechanism for its production[13]. Indeed, at that time, it is the only mechanism demonstrated to produce periodic color dots patterning in angiosperms[14 15] . Turing pattern relies classically on reaction and diffusion, and it seems that nervures could act as constraining semi-permeable barriers, which therefore shape the pattern into square-like shapes. Two clues suggest that nervures are semi-permeable to the diffusing agent (whatever its nature). First, no motif overlaps two nervures, indicating an impeded diffusion[16 17]. But if nervures were totally impermeable, there is no reason that motifs perfectly alternate from one 'line' to the next one, which is what we observe in every *Fritillaria* species. The most reasonable hypothesis is the possibility for a signal to diffuse to a long range, across nervures.

To sum up, to produce a final checkerboard-like patterns, several mechanisms need to interact: first, the lateral sides of the squares are produced by the straightness and parallelism of the tepal nervures. Secondly, the two other sides are more or less straight, depending on the species, and might depend on the Turing parameters of the system. Finally, the same Turing-like mechanism seems to be involved to produce an alternation of colored and uncolored zones, characteristics of the checkerboard-like patterns. This is summarized into a flowchart (Figure 4.A).

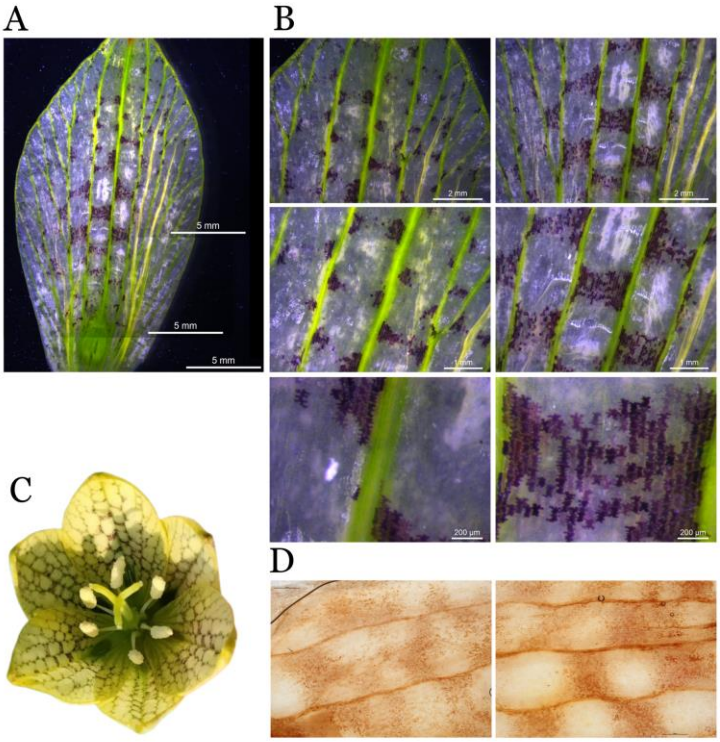

Figure 2. Fritillaria sp. checkerboard-like patterns. A and B: details of Fritillaria thunbergii tepal color patterns. C: Overall flower view. D: light microscopy views of Fritillaria meleagris checkerboard patterns.

Sarcophaga *checkerboard patterns are relying on a structural coloration generated by planar polarity of hair, compatible with the interference between two stripe patterns*

To explore *Sarcophaga* checkerboard-like patterns, we use several museum specimens of several species in the *Sarcophaga* genus. First, we observe the very peculiarity of these patterns. While it may appear as a black and white squares alternation, the reality is more complex. First, between two consecutive squares, there are tiny black zones, whatever the lighting. Then, under directional lighting, the black squares become light and vice versa depending on the angle of illumination and observation (Figure 3.A). These color changing patterns might then rely at least partially on structural coloration instead of only classical pigments. This was even more strongly suggested when specimens were recovered by a thin layer of platinum for SEM microscopy. Most of the time, this is sufficient

to cover and hide pigment-based patterns. On the contrary, checkerboard-like patterns were still visible, so the thin layer of platinum that molded the structure underneath seems to have taken a role of structural coloration[18 19]. Under the SEM, we observed that the external tissues of the abdomen were covered by two types of hair, a big and sparse one, and small and coarse one. Only the latter is compatible with structural coloration, because of its micrometric scale [20]. By qualitatively describing in a first part the mean direction and length of the hair (Figure 3.B, third panel), we observe a perfect correlation between the characteristics of the hair and the colors. The small black gaps between two squares are made up of tiny hairs, with not strong preferential direction, which give a glimpse of the structure underneath. On the contrary, color-changing areas correlate with dense hair with a strong preferential direction. Furthermore, if we compare two areas with opposite coloration behavior, (i.e. one is light when the other is black under a particular illumination angle), the hair length is similar, but their direction is symmetrical in relation to the right-left axis of the abdomen. Together, this strongly suggests that hair is responsible for the color behavior of the abdomen, through planar polarity[21].

Then, to explain the organizing into squares, we involved two observations: first, if we look at the thorax of the *Sarcophaga* flies, it is characterized by a striped pattern, parallel to the left-right axis[22]. If we hypothesized that this mechanism of stripe patterning is also present in the abdomen, it could explain alternations of the checkerboard-like pattern parallel to the antero-posterior axis. In the perpendicular direction, we observed that there are exactly two alternations per abdomen segments. Many insect species including many flies possess a bicolor coloration in each abdomen segment [23 24], one color on the anterior side, and the other on the posterior side. If we assume that this is also the case for Sarcophaga flies, this could explain the alternations perpendicular to the antero-posterior axis. Finally, as coloration seems to be coded by the preferential direction of the hair, the combination of two perpendicular signals may explain this checkerboard-pattern. This is summarized into a flowchart (Figure 4.B).

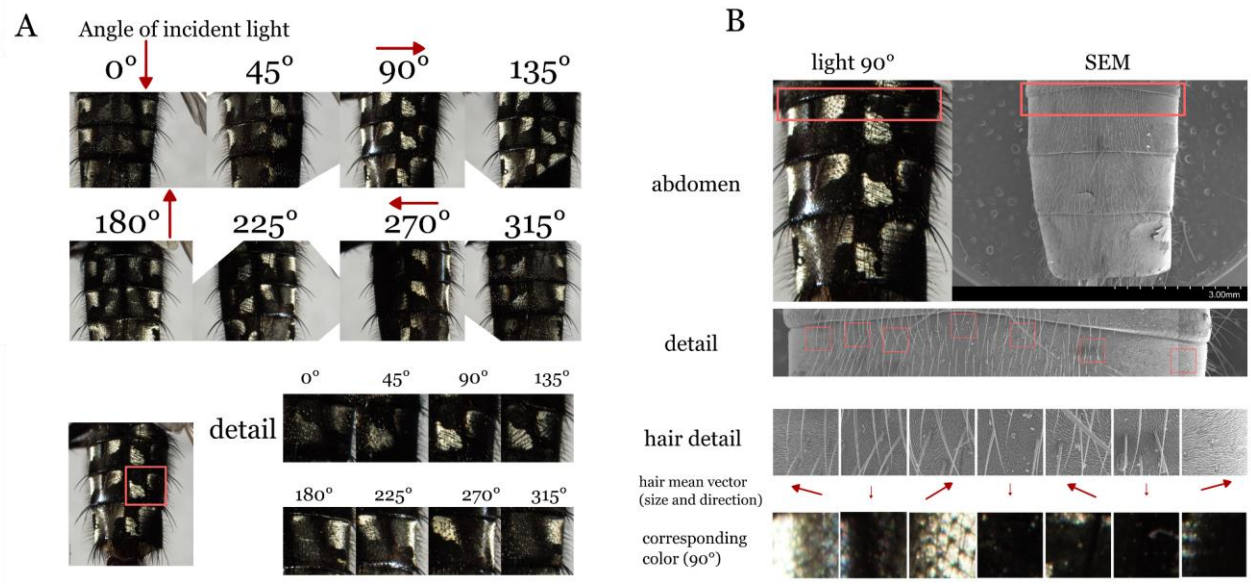

Figure 3. Checkerboard-like patterns of Sarcophaga sp. A: detail of Sarcophaga sp. abdomen color patterns depending on the incident light angle. Square color changes from dark to light depending on the light angle. B: upper panels: details of the abdomen under light microscopy (left) and SEM (right). Fro, up to down panels: detail of the most anterior segment, hair phenotype details, preferential direction and size vectors, color correspondence.

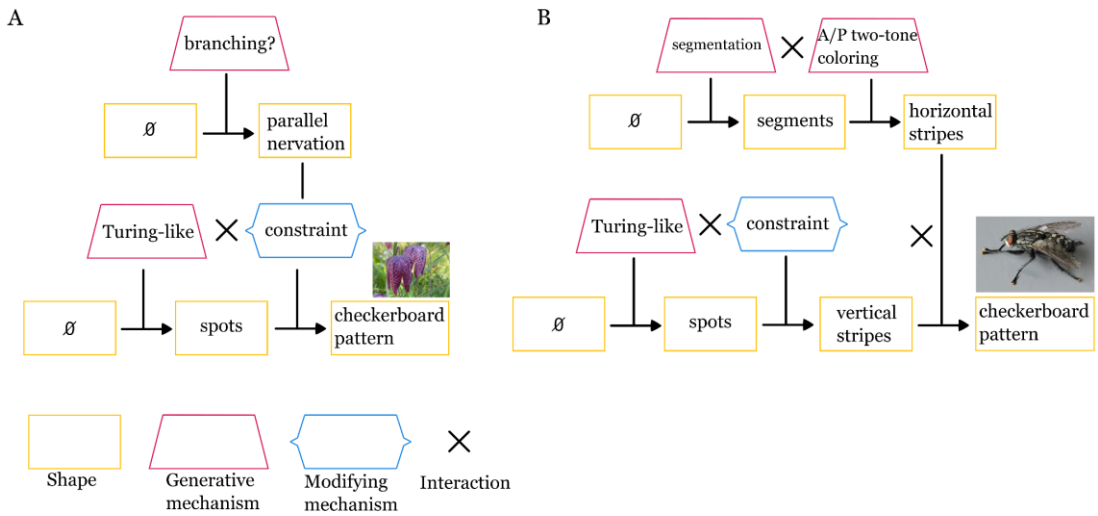

Figure 4. Morphogenesis flowchart of Fritillaria sp. (A) and Sarcophaga sp. (B) checkerboard-like patterns. Yellow rectangles: shape, pink trapezoid: generative mechanism, blue shape: Modifying mechanism.

Material and methods

Species survey and phylogenetic tree

The sources of photography were multiple, scientific literature was preferred, then online encyclopedias, Wikipedia (https://en.wikipedia.org/) and Wikimedia Commons (https://commons.wikimedia.org), eventually alternative sources (through search engines) with keywords such as "checkerboard", "checkered", "squares", “fritillaria”.

Phylogenetic relationships between species were built with phyloT (V2, https://phylot.biobyte.de/) based on NCBI taxonomy. Phylogenetic trees were plotted and annotated in iTOL (Interactive Tree Of Life, version 6.8.1, https://itol.embl.de/) [25].

Scanning Electron Microscopy

A total of three abdomens were sampled from *Sarcophaga* sp. museum specimens (National Museum of Natural History, Paris, France). Samples were mounted on the aluminum stubs with colloidal graphite, sputter-coated with platinum using an EM ACE600 fine coater (Leica, Wetzlar, Germany), and observed using a SU3500 scanning electron microscope (SEM, Hitachi, Tokyo, Japan).

Light Microscopy

A total of 20 tepals were sampled from 8 specimens of *Fritillaria thunbergii*, harvested in the botanical gardens of the University of Tokyo (Koishikawa Botanical Gardens, Tokyo, Japan). Tepals gas were extracted by a vacuum pump to make the tissues more transparent and suitable for observation. Abaxial and adaxial sides were mounted in water and observed under light microscopy.

A total of 8 tepals were sampled from 3 specimens of *Fritillaria meleagris*, from museum specimens (National Museum of Natural History, Paris, France), after a survey of hundreds of museum specimens. They were rehydrated and observed under light microscopy.

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