Title: Fractal geometry of a complex plumage trait reveals bird's quality

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Running head: Fractal geometry and plumage ornaments
Abstract:

Animal colouration is key in natural and sexual selection, playing significant roles in intra- and inter-specific communication because of its linkage to individual behaviour, genetics and physiology. Simple animal traits such as the area or colour intensity of homogeneous patches have been profusely studied. More complex patterns are widespread in nature, but they escape our understanding because their variation is difficult to capture effectively by standard, simple measures. Here we used fractal geometry to quantify inter-individual variation in the expression of a complex plumage trait, the heterogeneous black bib of the red-legged partridge (*Alectoris rufa*). We show that a higher bib fractal dimension predicted better individual body condition, as well as immune responsiveness, which is condition-dependent in our study species. Moreover, when food intake was experimentally reduced during moult as a means to reduce body condition, the bib’s fractal dimension significantly decreased. Fractal geometry therefore provides new opportunities for the study of complex animal colour patterns and their roles in animal communication.

Keywords: communication; condition-dependence; fractals; honest signals; immunocompetence; ornaments
1. INTRODUCTION

Colourful scales, feathers, furs or skins are often used by animals for camouflage and communication and play key roles in many natural and sexual selection processes [1-3]. To study these processes, we need to accurately measure trait variability, and study how it relates to fitness. In many cases, the trait of interest is too complex to be easily and accurately described by simple approaches. This is particularly true for patterns whose variability lies not only in the colouration or size of the patches, but in the spatial distribution and shape of colours across the body. Spotted, stripped, and other heterogeneous patterns are commonly found in the animal kingdom, showing different shapes, sizes, colours and distribution of their constituent units. Several methods have been developed to summarize and analyze trait colour characteristics, even considering the particularities of the observer visual system [4, 5]. However, only recently some techniques have been developed to describe spatial arrangement and patterning of complex colour patches [6-9] (table 1). Here we propose that fractal geometry provides a simpler method that can be easily applied to many animal colour patterns, providing an integrative measure that captures the complexity of a whole pattern when explored at different scales, which would be of great help to study their variability and functionality.

Fractal dimension (FD) was developed to describe self-similar mathematical objects, or fractals, whose shape is too complex to be described by Euclidean geometry [10]. FD is a highly integrative parameter whose value is influenced by properties such as number, length, tortuosity and connectivity of elements within a given object. Interestingly, many structures that we find in nature can be considered “statistical fractals” and their shape has often been successfully described by their FD [10-12]. In ecology and evolutionary biology, FD has proven useful for describing habitat structure
Here we propose that fractal geometry could be extremely useful to study the complex forms and colour patterns displayed by animals. Many of these complex colour patterns are fitness-related traits whose production likely requires coordinating processes at different scales to produce a coherent colouration pattern. Thus, fractal geometry might help to unravel information on the quality of the individual conveyed by these characters that could otherwise be difficult to assess with other methods.

We tested this idea using the red-legged partridge (*Alectoris rufa*) as a model species. Both sexes of this medium-sized bird display a conspicuous black bib, which is eumelanin-based and characterized by a complex pattern of black arrow-like patches against a white plumage backdrop [20, 21] (figure 1). This kind of melanin-based plumage trait is very common in birds and often used as a social signal [3]. Although the area covered by melanin is easy to quantify by digital photography [21], there is great variability in pattern and shape (figure 1b), which is much more difficult to capture. Because of the heterogeneous nature of the partridge’s black bib, we hypothesized that inter-individual variability could be well described by fractal geometry, and that FD could reveal hidden biological information conveyed by the trait expression.

2. MATERIAL AND METHODS

(a) Correlational study

42 six-month-old male red-legged partridges hatched and reared in communal outdoor pens were isolated in individual cages with water and food provided *ad libitum* [22, 23]. At the time of individual isolation all birds were weighted with a Pesola spring balance.
and their tarsus length measured with a digital calliper (±0.01 mm). For 24 of these males, we used the phytohaemagglutinin skin test [24] to measure immune responsiveness. 0.5 mg of phytohaemagglutinin (Sigma-Aldrich, ref. L-8754) suspended in 0.1 mL of phosphate buffer solution were injected in the patagium of the wing. The thickness of the patagium was measured three times before injection and 24 hours later with a digital spessimeter (Mitutoyo Absolute 547-315) to the nearest 0.01 mm. Both initial (r=0.99, F$_{23,48}$=510.3, $p$<0.001) and final wing web thickness measurements (r=0.99, F$_{23,48}$=336.2, $p$<0.001) were highly repeatable [25]. The difference between average initial and final measurements was used as index of cellular immune responsiveness [24]. We took digital photographs (2272×1704 pixels; Nikon Coolpix 4500 camera) of the breast of each bird under standard light conditions and bird position (the neck totally extended [21]), and keeping the bird-camera distance constant (40 cm). For each photo, the same standard grey reference and scale (Kodak Gray Scale, Kodak, New York, USA) was placed next to the bird’s neck.

(b) Experimental study

68 one-year-old birds (34 males and 34 females) were housed as for the correlative study during the moulting period, i.e. late June to mid November [20]. Before the moult (10$^{th}$ June), all birds were weighed and their bibs photographed as described above. Before the food restriction experiment (10$^{th}$ June), control and experimental birds did not differ in body weight (F$_{1,64}$=0.17, $p$=0.68), bib size (F$_{1,64}$=1.87, $p$=0.17) or bib FD (F$_{1,64}$=0.0, $p$=0.89), irrespective of their sex (non-significant sex×treatment interactions for all variables; all $p$>0.37). Cover feathers of the flange and breast were painted with permanent markers to later confirm that all birds completely moulted these plumage areas. For 20 males and 13 females, food provisioning was restricted during the
moulting period, so that their body mass was ca. 13% lower than controls (14 males and
21 females that were fed *ad libitum*) (see electronic supplementary material, figure A1).
The amount of food provided to food-restricted birds was continuously adjusted
according to our monitoring of bird’s body weight to create significant but reasonable
(i.e. within the range found in our captive population) differences between control and
experimental birds [22]. Body mass of all birds was recorded on 31st July, 21st August,
23rd September and 30th October (electronic supplementary material, figure A1). For
logistical reasons, only a subsample of 31 birds was weighed on 31st July. Our food
restriction protocol created the expected differences in body mass between control and
experimental birds throughout the moulting period (treatment×date effect on body mass:
F1,279=6.27, p=0.013; electronic supplementary material, figure A1) and similarly
affected males and females (non-significant treatment×date×sex interaction: F1,279=0.21,
p=0.65). Birds in poor condition exhibit narrower breasts due to reduced pectoral
muscle thickness [21], which could potentially affect our measures of bib size. To avoid
this potential methodological artefact, digital photographs of the bib after moult
completion were taken for each individual once both groups reached similar weights
(10th January). This was achieved by feeding all birds *ad libitum* after the moult
(treatment effect vanished at 10th January: F1,41=2.90, p=0.10).

(c) *Photograph analysis*

RGB values of all photographs were adjusted relative to those of the grey reference
placed next to the bird by using Adobe® Photoshop® CS3 (version 10.0.1). To do so,
RGB values of all pictures were equalized according to those of the grey reference
(R=G=B=160). Although linearization of RGB values was not performed here [26], this
is unlikely to affect our results because pictures were subsequently thresholded (i.e.
converted to black and white), and the black pattern of interest showed very high contrast with pale grey background colouration in the original images (figure 1). Bib size (i.e. the absolute surface area covered by melanin, in mm²) was measured by quantifying the area covered by black pigmentation using the “magic wand” tool of the same program. The processed images were subsequently used for calculating the FD using the box-counting method [10, 27] with the FracTop v0.3b software (http://seit.unsw.adfa.edu.au/staff/sites/dcornforth//FracTop/). Figure 2 illustrates how the box counting method quantifies bib’s FD. Repeatabilities, estimated from a subset individuals photographed on two consecutive days, were high (bib size: F$_{1,10}$=30.2, $p<0.001$, r=0.94; bib FD: F$_{1,10}$=8.9, $p<0.001$, r=0.80).

(d) Statistical analyses

For correlations, we used General Linear Models implemented in SAS 8.01 [28], testing whether bib size and FD predicted individual body condition or cellular immune responsiveness. For body condition, the dependent variable was the log$_{10}$(body mass), with log$_{10}$(tarsus length) as a fixed effect to control for structural size variation [29]. When entered as a fixed factor, body condition was estimated as the standard residuals of the regression of log$_{10}$(body mass) against log$_{10}$(tarsus length). Bib size and FD were entered as fixed effects. The bib’s FD positively correlated with total bib size ($r=0.53$, $p<0.001$, n=42). Therefore, to avoid multicollinearity issues, we ran the models either with bib size or FD as fixed effects and computed AICc differences between models ($\Delta$ AICc) in order to compare how well different models predicted body condition or cellular immune responsiveness [30]. If bib's FD model performed better than the one with bib size it would mean that despite both variables are correlated, bib's FD was better predictor of the dependent variable. For the experiment, we used General
Linear Mixed Models with individual identity included as random factor. Body mass, bib size or bib FD were considered as dependent variables, whereas sex, sampling time (before vs. after moult) and treatment (control vs. food-restricted) and all their interactions were entered as fixed effects. Given the mentioned relationship between bib size and FD, bib size was entered as covariate in the model for bib FD. Full models for the effect of experimental manipulation on bib size and bib FD are given in table 1. All tests are two-tailed and means or slopes are given ± s.e.m. Data are deposited in the Dryad repository (http://dx.doi.org/10.5061/dryad.83873).

3. RESULTS

(a) Correlational study

Bib FD predicted individual body condition ($F_{1,39}=13.7$, $p<0.001$, slope±s.e.m.=0.47±0.13, whole model adjusted $R^2=0.36$). Bib size also predicted body condition ($F_{1,39}=5.36$, $p=0.02$, slope=0.32±0.14, adjusted $R^2=0.24$), but the model was worse than the one including FD as explanatory variable ($\Delta AICc=7.2$). Bib’s FD also predicted cell-mediated immune responsiveness ($F_{1,22}=17.4$, $p<0.001$, slope=0.66±0.16, adjusted $R^2=0.42$), whereas bib size did not ($F_{1,22}=2.88$, $p=0.10$, slope=0.34±0.20, adjusted $R^2=0.07$). Accordingly, bib’s FD model performed better than bib size model ($\Delta AICc=11.0$). Body condition and cell-mediated immunity were positively associated ($F_{1,22}=25.5$, $p<0.001$, slope=0.73±0.14, adjusted $R^2=0.52$), and body condition predicted cell-mediated immunity better than FD ($\Delta AICc=4.4$). Therefore, the association between the bib’s FD and immune responsiveness might be mediated by condition. Indeed, when body condition was added as a covariate, the relationship between bib FD and cell-mediated immunity became non-significant ($F_{1,20}=1.37$, ...
Irrespective of the mechanism involved, these results indicated that fractal geometry provided a simple measure of the complex pattern that revealed biologically meaningful information about the bearer’s quality.

(b) Experimental study

In order to confirm our correlative results, we restricted food access throughout moulting period to a group of 43 partridges so that their body condition was significantly lower than that of a control group (n=35) that was fed *ad libitum*. Bib size increased after moult in all birds, and similarly in control and treated birds (figure 3a and table 2). In contrast, experimental reduction of body mass reduced bib’s FD in both sexes (significant time×treatment interaction, but not significant sex×time×treatment interaction; figure 3b and table 2): in controls, the bib’s FD did not change significantly (F1,33.1=0.18, p=0.68) whereas the bib’s FD was significantly reduced in birds that experienced food restriction during moult (F1,35.7=18.2, p<0.001; figure 3b). We therefore confirmed a causal relationship between body condition and FD, evidencing that fractal geometry captures quality-related information codified in an animal colour pattern that would remain unnoticed otherwise.

4. DISCUSSION

By combining correlational and experimental evidence, we have shown that fractal geometry can reveal biologically meaningful information encoded in a complex plumage trait, the black spotted bib of the red-legged partridge. Our correlative results indicate that both better condition and greater immune responsiveness can be predicted from bibs with higher FD. Given that individuals in better condition had greater
immune responsiveness to PHA, a mediating effect of condition in the relationship
between bib FD and immune responsiveness is likely. Both immunocompetence and
body condition are indicative of individual quality, and were better predicted by bib FD
than by bib size alone. In addition, the condition-dependence of bib FD was supported
by experimental evidence: when individual body condition was experimentally
worsened during moult, new bibs showed a lower FD than bibs previously displayed by
the same birds, while control (ad libitum fed) individuals moulted bibs with a similar
FD. Therefore, the fractal properties of the plumage trait were dynamically updated
according to bird body condition during moult, thus potentially being an honest signal
in communication scenarios.

The FD provided a simple measure (one variable) of the complex trait’s pattern
that was more informative about condition or immune responsiveness of the individual
than the total bib area alone. But, what does FD tells us about bib morphology? Natural
fractal objects are heterogeneous objects that behave (statistically at least) similarly at
different scales [10]. That is, they do not show sharp transitions when one gradually
zooms in and out of the object. The black bib of the red-legged partridge is composed
by an upper solid black area that turns into a series of spots that spread through the chest
of the bird (figure 1). For the partridges’ bib, the FD may accurately describe the
smoothness of the transition between the plain and spotted areas of the bib (figure 1a).
Accordingly, once taking into account the total pigmented area, those bibs with
relatively higher FD are those characterized by a smooth transition between the uniform
black throat patch and the lower spots (figure 1b). In contrast, those bibs with relatively
smaller FD (for a given bib size) showed a sharp discontinuity between the solid black
collar and the spotted lower part of the bib (figure 1b). What makes measuring FD
particularly interesting for bird colour patches or other complex animal patterns is that
FD not only captures the traditional way of quantifying these traits (e.g. total size or surface area, figure 1), but also improves the quality of the information by adding a measure about the internal structure of the colour patch. However, the specific information conveyed by FD should be explored in each case, as we have done for our study pattern. Note that even negative correlations between FD and a fitness trait may also be expected; e.g. if a simpler colouration pattern such a well-defined striped patch is more difficult and costly to produce than a more noisy and complex pattern.

The study of pattern components has been neglected compared to analyses of patch colouration. Apart of attempts to quantify the entire colour pattern of animals considering the relationships among the colours of an individual [5], methods to quantify the shape and geometry of these colour patterns have been explored only recently. These methods are briefly described in table 1. FD can potentially be applied to any kind of pattern (spotted, barred or irregular shapes) and provides a synthetic description of a patch’s appearance. Another interesting feature of FD analysis is its simplicity of calculation: FD can be easily computed from digital images, requires minor image processing and can be obtained from a variety of freely downloadable softwares easy to use (e.g. Fractop, HarFa, ImageJ). However, one potential limitation is that FD may sometimes be difficult to interpret. Given that it results from a combination of several independent features (i.e. proportion of area pigmented, size, shape, location or connectivity of colour markings), identifying what aspect/s of the pattern is/are actually producing the results may require further exploration, as we have done here (figure 1b). In any case, whether FD relates (and if so, to what extent) to the indices provided by methods listed in table 1 could be explored in the future. This will help to determine the most appropriate combination of indices to better describe a given colour pattern.
Our results open up a new research window for the study of complex animal traits or to unravel new aspects of simpler ones. Colour patterns are the result of a tight control over the expression of multiple mechanisms that must be synchronized at very different scales (from pigment synthesis and deposition into a single feather, scale or hair, to the coordinated growth and distribution of these units along the entire body). Factors affecting developmental stability [31] may alter this machinery, resulting in changes in the FD of the trait. FD has precisely the virtue of measuring the continuity of a pattern through scales. This property makes FD an interesting tool to capture the variability in shape and structure resulting from the above mentioned multi-scaled construction of many animal traits, which is particularly relevant for the study of honest (costly to produce) animal signals. Also, other possible applications of FD may not imply an intrinsic positive or negative fitness value of this variable, which may in turn be used as a neutral descriptor to capture and summarize the appearance variability between individuals, morphs, populations or taxa [32, 33]. There are multiple potential applications of fractal geometry to extract meaningful information from complex animal patterns, and future studies should further explore the usefulness as well as the possible shortcomings of this promising tool.

But, apart from the methodological insights, our work claims for further studies on fractal perception in animals, depending on their visual processing abilities. The only requirement for FD to convey information available to the receiver is that differences in pattern appearance captured by FD are actually detectable by a specific animal visual system. Studies in animals addressing this issue are currently lacking. In humans, however, studies have shown that the FD of artworks unconscientiously influences our perceived beauty and preferences, be they purely abstract designs or realistic representations [34-36]. Non-human animals may similarly prefer traits with higher (or
lower) fractal dimensions, particularly if these advertise a better individual quality, as
we report here. We therefore suggest that considering FD should shed new lights onto
the evolution and maintenance of complex animal patterns.

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current laws.

REFERENCES


Table 1. Summary of the main capabilities and limitations of the available methods to quantify pattern appearance, including the use of fractal dimension proposed here.

<table>
<thead>
<tr>
<th>Method</th>
<th>Information provided</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>An index of within-pattern luminance contrast can also be obtained.</td>
<td></td>
</tr>
<tr>
<td>Granularity analysis [7, 8]</td>
<td>A granularity spectrum is obtained for each pattern, allowing obtaining independent descriptors of marking size distribution and degree of contrast between markings and background.</td>
<td>Requires programming skills and complex image processing. It does not provide information about the shape of the markings or their connectivity.</td>
</tr>
<tr>
<td>Colour adjacency [9]</td>
<td>Allows calculating independent indicators of relative colour frequency, pattern regularity (i.e. transition density) and pattern elongation (i.e. aspect ratio).</td>
<td>Requires programming skills and complex image processing.</td>
</tr>
<tr>
<td>Fractal dimension (FD) [this study]</td>
<td>A single index (FD) captures variability in trait appearance integrating different aspects of pattern shape across scales.</td>
<td>The specific aspect/s of the pattern appearance contributing to FD must be explored case by case.</td>
</tr>
</tbody>
</table>
Table 2. Effect of the experimental reduction of body condition during moult on partridges’ bib size and fractal dimension. Significant effects are highlighted in bold.

<table>
<thead>
<tr>
<th></th>
<th>Bib size</th>
<th>Bib fractal dimension</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>F</td>
</tr>
<tr>
<td>bib size</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>sex</td>
<td>1, 64</td>
<td>30.2</td>
</tr>
<tr>
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<td>sex×treatment</td>
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<tr>
<td>time×treatment</td>
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<td>2.93</td>
</tr>
<tr>
<td>sex×time×treatment</td>
<td>1, 64</td>
<td>1.02</td>
</tr>
</tbody>
</table>

(Sex, time -“before” or “after” molt- and treatment -“control” or “food restricted”- were entered as fixed factors in both General Linear Mixed Models, whereas bib size was entered as a covariate in the first model. Individual was entered as a random term in both models).
Legends for figures

Figure 1. (a) Male red-legged partridge displaying its black bib (photo credit: Hans Hut). (b) Relationship between the fractal dimension (FD) and size (mm$^2$ of pigmented area) of the black bib. Bibs of similar size but with high and low FDs (above and below, respectively) are shown for a range of bib sizes. For a given bib size, bibs of higher FD consistently show a smooth transition between the uniform black throat patch and the lower spots whereas bibs with relatively smaller FD show a sharper discontinuity between the solid and the spotted parts of the bib.

Figure 2. Example of calculation of bib fractal dimension (FD) using the box-counting method. The black and white image of the bib (a) is overlaid by meshes of different cell side lengths (e.g. $s=128$ pixels) and the number of cells occupied by at least one black pixel is counted for each mesh size (e.g. $N=18$). This results in the dataset (b). Plotting Log($s$) vs Log($1/N$), we estimate the FD of the bib as the slope of the fitted straight line, e.g. FD=1.794 (c). Note that FD captures how the number of boxes containing the plumage pattern changes when analysing the pattern at different scales (i.e. when changing cell side length).

Figure 3. Changes in the mean (±s.e.m) (a) size and (b) fractal dimension (after controlling for bib size) of the bib of red-legged partridges that were fed *ad libitum* (control, n=35) or kept under food restriction (n=33) during moult.
Figure 1

(a) [Image of a bird]

(b) [Graph showing fractal dimension vs. bib size (mm²)]

Fractal dimension

Bib size (mm²)
Figure 2

<table>
<thead>
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<th>Log(s)</th>
<th>Log(1/N)</th>
</tr>
</thead>
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<td>30131</td>
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<tr>
<td>128</td>
<td>18</td>
<td>4.8520</td>
<td>-2.8904</td>
</tr>
</tbody>
</table>

$s = 128 \quad N = 18$
Figure 3

**a**

- **Bib size (mm²)**
- **control**
- **food restricted**

**b**

- **Residual fractal dimension**
- **Before moult**
- **After moult**

- **Y-axis:** Residual fractal dimension
- **X-axis:** Bib size (mm²)