Format for the research paper:

Write this as if you were submitting it as a scientific body of work to be peer reviewed prior to publication. Model it after a standard research paper, with an abstract, introduction, results/discussion, conclusions/future work, references, figure captions, and figures (in that order). The paper should contain a title page (with title, authors and addresses, and abstract), approx. 5-7 pages of text (double-spaced, one side only) a page containing properly formatted references, a page containing figure captions (single-spaced captions, with double spacing between captions), and at least 2 figures (one per page).

To remind you, the project grading will be as follows:

Statement of Intent:	5pts.
Proposal:	25 pts.
Paper:	30 pts.
Poster:	20 pts.
Total:	80 pts.

Formatting and writing:

1. Abstract: The first sentence should state what was generally investigated and the principal methods used. Then, summarize the key findings.
   Introduction: review of information already available in the literature to place your work in context.
   Experimental: description of instruments and instrumental methods (e.g., sample preparation, reagent sources, etc.)
   Results/Discussion: always present the results first, then interpret them, in that order.
   Conclusions: brief, one paragraph summary of key findings and possibly a statement about future work.
2. Tables and Figures: Tables and figures are generally presented last in the manuscript, with tables coming before figures. Tables are generally numbered with capital Roman numerals and figures with standard Arabic numerals. 3. The manuscript should be written in the past tense when describing experiments and results.

Figures

1. Data points are always represented by discrete symbols with error bars. Theoretical curves and/or fits are always represented by lines.
2. If connecting data points, always do so using straight lines between adjacent points (e.g., not a spline plot). The one exception is if you are doing a curve fit to an equation with a known functional form. In any case, do not extend the fit beyond the range of the acquired data.
3. Every principal author will have their own preferences, but for this class the figures in manuscripts should generally be 4” by 5” (w by h) and printed using portrait orientation (not landscape). This format reproduces well in journals using two-column text formatting.
4. When using color in the manuscript figures, be sure that the figure can still be meaningfully interpreted when reproduced in black and white (e.g., use both color and line-style to

differentiate curves). Following publication, you should anticipate most people photocopying or printing out your article will do so in black and white only.
6. Never include the equation for a linear fit on the figure (y=mx+b) unless you plan to define each term. Instead, report the slope and intercept with errors and appropriate units.
7. Most journals will not accept simple calibration curves in scientific manuscripts.

Before you submit your proposal, check the things that especially bother Garth in scientific writing:

1. <u>Every paragraph must start with an appropriate topic sentence that encompasses all material described in the paragraph.</u>
2. Unless you're talking about the car, "spectra" is a plural noun and "spectrum" is singular.
3. "Data" is a <u>plural noun</u>; datum is the singular but it's more common to use "data point".
4. Dangling "this". "This" should never be the subject of the sentence since it introduces ambiguity. For example, instead of "This is silly", write "This rule is silly", etc.
5. When used as a preposition, "that" should never be preceded by a comma and "which" should always be preceded by a comma (Word's grammar-check will catch this one).
6. Always run spell-checker and grammar-checker before submitting your work.
7. Never ever ever never ever use "I" in writing or speaking and minimize the use of "we" in scientific writing.
8. Anything named after a person should be capitalized, but otherwise do not capitalize. For example, "Gaussian distribution (GD) versus normal distribution (ND)".
9. "e.g.," means "for example" (exempli gratia), and "i.e.," means "in other words" (id est).
10. Never use "et al." (et alii, meaning "and others") in the references. It is ok to use it in the text body when referring to a specific article with multiple authors (first author's last name followed by et al.). If you are referring to multiple citations from the same group, use instead "XXX … and coworkers". Also, note that "et" is a complete word and only "al." is an abbreviation.
11. Never use "deals with".
12. Avoid absolute and subjective descriptions, such as "only, none, every, best, worst, very, etc." There are no such things in science.
13. A comma is placed before "and" or "or" if it is used to separate two complete independent clauses containing subjects and verbs, and it is not included if the second clause lacks a subject.
14. Do not use the word "setup" when describing experimental apparatus.
15. The plural of "apparatus" is "apparatus".
16. Keep references to websites to an <u>absolute minimum</u> since these can disappear at a moments notice!! References to publications carry much more weight. On the other hand, if you do not include a reference, it had better be original artwork or it will be dismissed on the grounds of plagiarism! If you are planning to include information from the web in your proposal, try to find similar information/figures in published material instead.

Electrophoretic Separation of Bird Mixtures: Direct Evidence for a Correlation between Feathers and Flocking-Togetherness

L. J. Campbell, B. P. Lynch, N. J. Begue, V. A. Shubert, and G. J. Simpson*

Department of Chemistry, Purdue University
West Lafayette IN 47907-1393*

Electrophoretic separation with fluorescence detection was performed on several bird populations to investigate correlations between and properties of birds and the environment from which they were extracted. Two-dimensional electrophoresis measurements combining isoelectric focusing and sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) performed on standard bird samples indicated that birds of a feather flocculate together. Subsequent analysis of native Hawaiian bird populations indicated that in some instances (but not all), birds of feathers also flock together, providing the first direct experimental evidence supporting the premise "birds of a feather flock together." Interestingly, the sex and age of the birds also influenced flocking behavior, indicating a statistically significant abundance of mother-flocking birds.

*Author to whom correspondence should be addressed.

Introduction

The statement "birds of a feather flock together" is a well-known and widely used axiom.[1] In spite of its nearly universal usage, little scientific evidence has been presented to support or refute this assertion. A major limitation of these previous studies has been the reliance on empirical observation to identify and quantify the various bird populations and densities. The few isolated quantitative studies that have appeared in the literature have produced conflicting results.[2-6] For example, Snuffalufagus *et al.* claimed to have validated the axiom by employing capillary zone electrophoresis / mass spectrometry (CZE-MS) in analysis of matrix-matched bird samples from the deep Amazon.[5] However, the results of this study were later recanted after it was discovered that one of the researchers (Bird, B.) had apparently inadvertently contaminated the samples.[6]

In order to overcome many of these impediments, an independent analysis method is required to allow quantification of each bird species through a characteristic species-specific parameter. One intriguing possibility is the use of differences in the electrophoretic mobility of birds to identify and quantify bird populations in a timely and noninvasive manner. The movement of charged particles in static electric fields has been exploited extensively for separation and identification of macromolecules.[7] Recent extensions of that work to include studies of live cells suggests the exciting possibility of working with even larger living systems.[8] In this work, electrophoretic separation was used to directly probe the flocking behavior of native bird populations.

Experimental

Electrophoretic separation of bird mixtures. A series of known bird standards (Lafayette Petsmart) was suspended in a low-conductivity buffer solution (30 mM HEPES and 100 mM sucrose). Using Bird-O-Fluor3 (Molecular Probes), the birds' external appendages were fluorescently labeled to facilitate bird detection. Two-dimensional (2D) electrophoretic separation was performed on a plug of each bird suspension, introduced into a well in the gel. The bird sample was then separated using applied static electric fields ranging from 1-3 kV/cm, as shown schematically in Figure 1. Isolelectric focusing was performed with pH gradient across the acrylamide gel, followed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). The bird samples were kept sufficiently far from the separation electrodes to ensure they would not be harmed during the procedure. Fluorescence imaging of the gel after separation allowed for determination of the concentration of each bird type present in the original sample.

Field studies of unknown samples. Field investigations were performed in thriving ecosystems in and around the island of Hawaii. Sample collection and preparation were critical considerations. Initially, sample collection was performed using a method first introduced by Hitchcock in 1963, depicted in Figure 2.[4] However, complications with this method included driving the analyte birds into fits of murderous rage. To reduce the general administrative inconvenience associated with graduate student fatalities, the standard Hitchcockian method was modified to include an initial separation and preconcentration step that minimized the potential for avian-human conflict. The distillation scheme for achieving sample collection and preconcentration is depicted in Figure 3. Each initial sample of forest was collected and placed into a 500 ML beaker. This large beaker was attached to a heptaliter (10 ML) aviary nesting device (HAND), by a wide-diameter tube (I.D. = 3 m), and the entire system was hermetically sealed. Coordination of efforts between multiple individuals separated by large distances during these preconcentration steps was facilitated through a system of simple hand gestures, referred to as giving one another the bird.[9] The higher vapor pressure of birds compared to trees led to fairly rapid equilibration (within a few days) between the bird populations in the two containers, with virtually no tree or bush component in the HAND. Because of preferential partitioning of birds into the beaker containing foliage, the equilibrium concentration of birds was approximately two birds in the bush for every one in the HAND. Following distillation, the HAND was removed, and its contents were suspended in a phosphate buffer solution and analyzed. Following data acquisition and analysis, the birds were returned to their natural environment.

Results and Discussion

A Hitchcock plot acquired during the bird distillation is shown in Figure 3. The characteristic Hitchcock time constant τ_H describes the rise and plateau to maximum bird concentration during the distillation (Figure 4). The many twists and turns evident in Figure 4 are characteristic of Hitchcock plots.[4] An unintended spike in the bird concentrations above the Bates Plateau[10] resulted in the deaths and/or maimings of several researchers, as described in the Experimental section, and should generally be avoided.

Prior to analysis of native bird populations, a series of standard samples was suspended in low-conductivity buffer solutions to generate bird calibrations curves. Although there was some variation across specie, the instrument consistently yielded linear dynamic ranges spanning approximately four decades, with bird detection limits in the attomole range.

Following instrument characterization and calibration, 2D electrophoresis measurements were performed on Hawaiian bird samples. Bird samples were collected using the modified Hitchcockian avian fractional distillation method as described in the Experimental section. The 2D electropherograms for a representative standard and sample are shown in Figure 5. Measurements were also performed on isolated samples of four different classes of birds: adolescent (less than 1 year old) females, adolescent males, mature females, and mature males. Compared with the other three classes of birds, a statistically significant propensity for flocking was observed in the population of mature females, which will be referred to as mother-flockers.

The 2D electrophoresis measurement of bird standards revealed some interesting trends. The distance traversed during the SDS-PAGE step of the electrophoretic separation is indicative of the bird "size", or more precisely the weight and obesity / restricted mobility (WORM) factor.[11] The larger and denser birds eluted more slowly during the SDS-PAGE separation, and their motilities were not consistent with the WORM model. In contrast, the smaller birds eluting further and earlier were well described using the WORM approach, indicating a clear correlation between early birds and WORMs.

Analysis of the gel electropherograms represented in Figure 5 indicated another intriguing correlation between different bird properties. Looking down a column (representing SDS-PAGE separation), most columns contained only a single fluorescent spot. This aspect of the separations suggests that the isoelectric focusing yielded a single well-defined bird population at each pH. Given that a bird's electrophoretic mobility is often dominated by the charge characteristics of its feathers,[11] these results suggest that birds with the same feathers flocculate together.

Measurements of multiple native Hawaiian bird populations provided some evidence for correlated bird concentrations (i.e., flocking behavior). Statistical treatment of the results from 27 different samples produced distributions in bird populations that could not be described assuming uncorrelated populations. Furthermore, significant variations were observed across bird types. For some specie, flocking behavior was clear, while for others little if any evidence for flocking was present. Although the limited number of samples did not allow for unequivocal elucidation of the underlying mechanism behind bird flocking, these results are the first to our knowledge that provide direct evidence confirming that in some instances birds of a feather do flock together.

Conclusions

Two-dimensional electrophoresis measurements of standard and native bird populations demonstrated correlations between bird feathers, flocculation behavior, and populations in native samples. From the results of these flocking experiments, clear evidence was present supporting the axiom "birds of a feather flock together" in some specie, while in other bird specie no detectable flocking behavior was observed. Experimental studies on standard bird samples demonstrated what appears to be a general trend in isoelectric focusing of birds; specifically, birds of a feather flocculate together. Continuing studies in our laboratory are focused on answering the question: "Do urban and rural bird populations, being of similar feather, still flock together?" The potential influence of environmental conditions (e.g., rain pH, ozone levels, etc.) shall be examined in an effort to develop a more encompassing unified bird theory (UBT).

Acknowledgments

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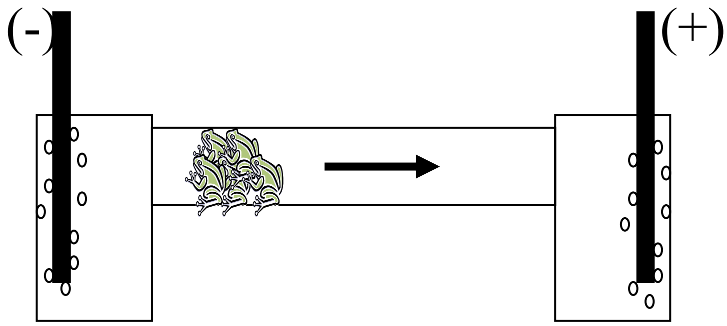

Figure 1. Schematic of the instrument used for bird electrophoretic separation. As indicated by the arrow, negatively charged birds will migrate toward the positive pole.

Figure 2. Avian samples after Hitchcock distillation and preconcentration (adapted from Ref. 4).

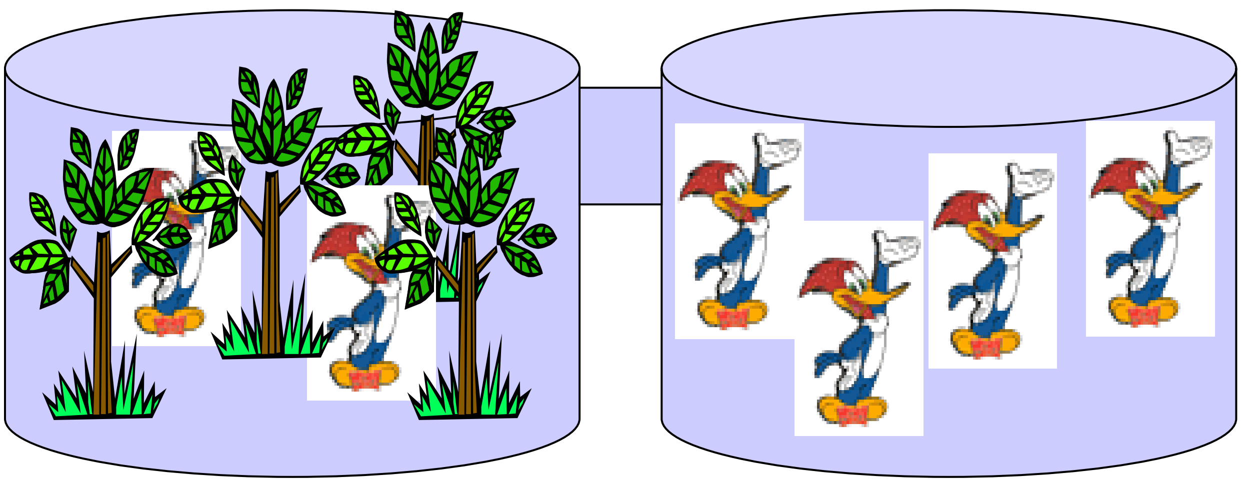

Figure 3. Schematic of the HAND apparatus employed for bird distillation and preconcentration.

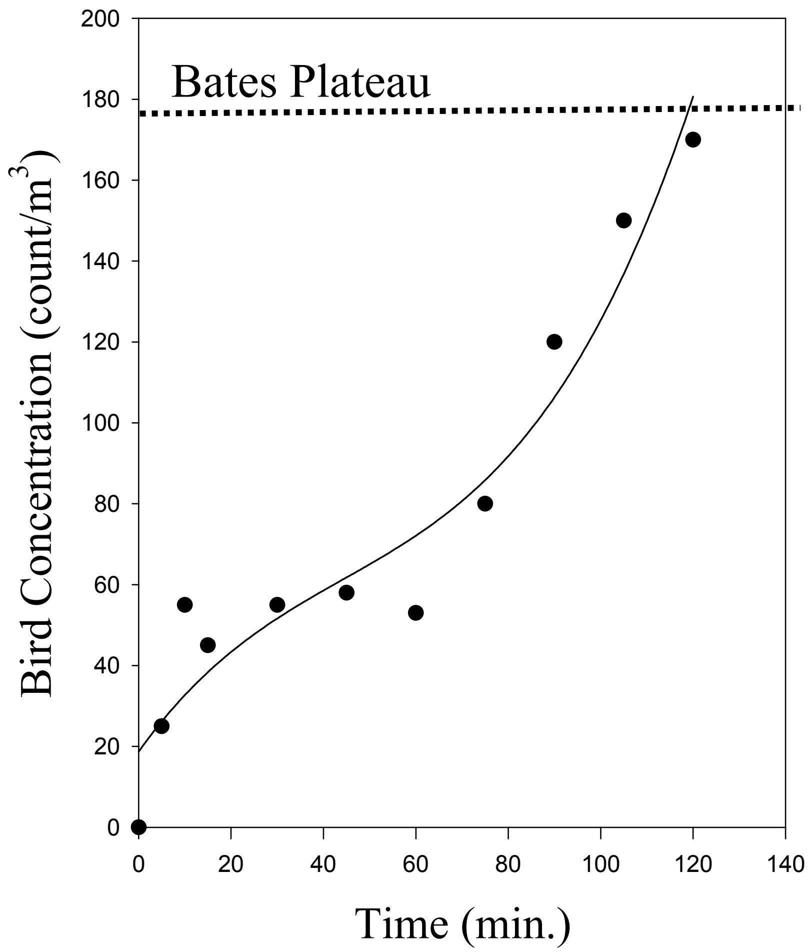

Figure 4. Hitchcock plot of bird distillation, indicating the time dependence of the bird population in the collection HAND.

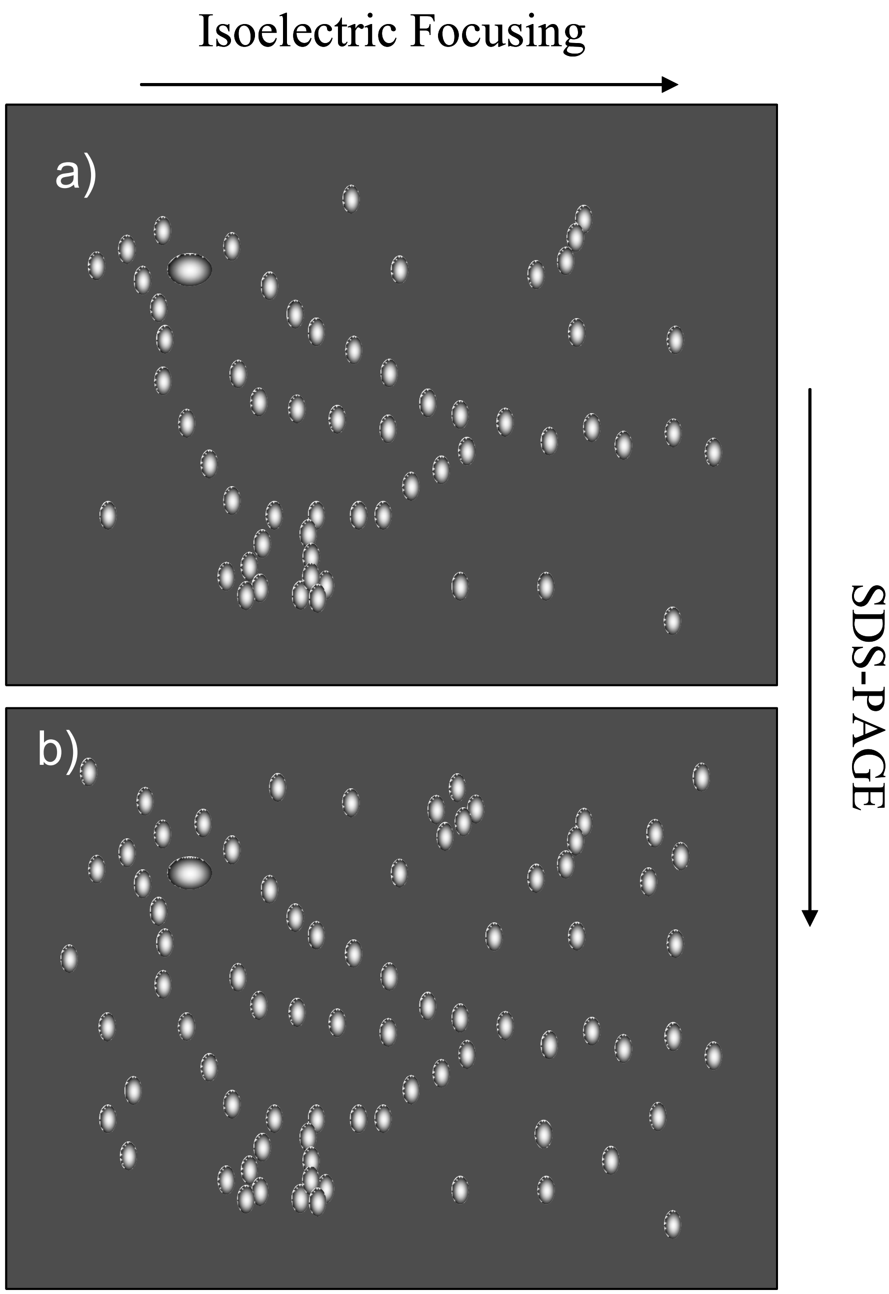

Figure 5. Representative two-dimensional electropherograms of avian standards (a) and Hawaiian bird samples (b). Bright spots indicate regions of high fluorescence. Isoelectric focusing was performed along the horizontal axis, and SDS-PAGE along the vertical axis.