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Evolution of Foraging Traits in Hummingbirds

By

Nicolas Marcel Alexandre

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Noah K. Whiteman, Chair

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Professor Robert Dudley

Fall 2022

Mechanisms of Trait Evolution in Hummingbirds

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By

Nicolas Marcel Alexandre

Abstract

Mechanisms of Trait Evolution in Hummingbirds

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Since the advent of animal-mediated pollination, pollinators have evolved adaptations to optimize their intake of floral rewards (1). The hummingbirds are one such group of pollinators that have diversified into over 300 extant species (2). A hallmark of hummingbirds is their extraordinarily long bills that also vary dramatically in curvature. The intertwined relationship between variation in hummingbird bills and the corresponding variation in flower shapes, colors, and sizes they feed from have resulted in a suite of morphological, physiological, and behavioral adaptations that we are just beginning to understand. In this dissertation, I gathered morphological and genomic data to address the hypothesis that rapid adaptation in response to environmental change continues to shape natural variation in foraging traits of wild hummingbirds of North America. In Chapter 1, I found that a growing human population size and the introduction of supplemental feeders and *Eucalyptus* into California during the 20th century is consistent with rapid shifts in population size of the Anna's Hummingbird, and has facilitated changes in bill size and shape associated with climate and resource density. In Chapter 2, I provide evidence that a novel form of ultraviolet vision occurs through pre-existing color vision mechanisms following a genomic deletion of the UV opsin in the Broad-tailed Hummingbird. Chapter 3 reveals that variation in floral preferences, bill morphology, and long-distance migration phenologies are linked in a population of the Broad-tailed hummingbirds at the phenotypic and genomic levels, and may provide a mechanism for subtle niche divergence and specialization between individuals in canonical foraging traits.

Dedication

This dissertation is dedicated to my mom and dad, without whom none of this would be possible in more ways than one.

Acknowledgments

I would first and foremost like to thank my mom and dad for supporting my dreams from the start, spending countless hours driving me to my various jobs at the crack of dawn and giving me more books than I could possibly read. I also want to thank my grandfather Ed for instilling a love for nature at a young age and giving me the opportunity to interact with it on a much deeper level. And my grandmother Barbara, who has always shared such a deep interest for science with me. To my brother Ryan, you always give me the most insightful pep talks and support me no matter what I do. I love you all.

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Author Contributions

Chapter 1

Faye Romero (co-first author) – Measurements and data analysis
Eliza Grames – Data generation for term counts of Eucalyptus and Hummingbird Feeder
Zoe Migicovsky – Advising and data analysis of geomorphometric data
C.J. Battey – Data generation of climate and bird survey data
Laura Stein – Advising on statistical analysis
Timothy Barnes – Photographic measurements
Saron Akalu – Photographic measurements
Haarini Sridhar – Photographic measurements
Gillian Montross – Photographic measurements
Alejandro Rico-Guevara (anchor author) – Advising, experimental design, thermal imaging

Chapter 2

Kirsten I. Verster – Synteny analysis
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Mary C. Stoddard – Advising, experimental design
David Inouye – Permits and Sample Collection
Sree R. Kolora – Bioinformatic advising
Manny Vasquez – Bioinformatic advising
Daniel S. Rokhsar – Genome assembly
Peter Sudmant – Advising on structural variant characterization
Manjusha Chintalapati – Advising on structural variant characterization
Noah K. Whiteman (anchor author) – Advising, experimental design

Chapter 3

Timothy Barnes – Photographic measurements, field assistance, library preparation
Faisal E. Alzaben – Data analysis and visualization
Faye Romero – Photographic measurements,
Irina Chen – Photographic measurements, pollen collection and identification, field assistance
Jimmy Lee – Photographic measurements, field assistance, experimental design
David Inouye – Permits and sample collection, experimental design
Mary Stoddard – Permits, experimental design, advising
Sree R. Kolora – Bioinformatic advising
Thorfinn Korneliussen – Bioinformatic advising
Zoe Migicovsky – Advising and data analysis of geomorphometric data
Haarini Sridhar – Photographic measurements
Gillian Montross – Photographic measurements
Tyler Lindereth – Bioinformatic advising
Shana McDevitt – Sequencing and library preparation
Rasmus Nielsen – Bioinformatic advising
Noah K. Whiteman (anchor author) – Permits, experimental design, advising

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Rapid Evolution of Anna's Hummingbird Bills via Anthropogenic Change

Introduction

Urban areas are epicenters of environmental change, affecting land cover, biodiversity, and the global climate (1). As humans have expanded across the globe, novel selective pressures have induced rapid adaptation of local species (2–4). Changes in resource availability in the form of supplemental feeding or housing developments have provided additional foraging and nesting opportunities to many native birds, supporting larger population densities and facilitating range expansions (5–7). Such changes have resulted in various examples of local adaptation, particularly with respect to bill size or shape, which have direct consequences for individual survival and fitness (4, 8). Other indirect effects of human activity, like climate change, also select for local adaptation to changing thermal environments and can facilitate rapid range expansion across environmental gradients (9, 10). While range expansion is often driven by both urbanization and climate change in non-uniform ways, the timing and degree of local adaptation during such expansions in urban-dwelling species remains unknown (5, 11, 12).

The Anna's Hummingbird (AH; *Calypte anna*) represents a prime example of a species at the confluence of these different forces and can be used as a model to study the modularity of phenotypic change in response to multiple interacting environmental changes. Since the early 20th century, AH have established a tightly intertwined relationship with humans. While historically limited to breeding populations in Baja and southern California, the AH range had expanded up through the Sacramento Valley as early as 1915, but its year-round range has now expanded north along the Pacific Coast of North America to southeastern Alaska (5, 13). An increase in population size was noted in 1930's and was thought to be due to the introduction of multiple species of *Eucalyptus* into California in the mid-19th century, many of which attract nectarivorous birds within their native Australian range (14, 15). The plausibility of this scenario can be attributed to the high nectar rewards, morphologically unspecialized (open) flowers, and a long flowering period from October to April (16). Since then, warming temperatures and urbanization are thought to have contributed to subsequent range and population size expansions of the AH in the 1990's, where feeder visitation in the expanded range has increased relative to usage in the historic range and colonization probability is higher where housing is most dense, suggesting a reliance on supplementary feeding and habitat microclimate (5). The population expansions in the early and late 20th century are therefore associated with distinct changes to the environment, but no systematic analysis of their relative contributions have been assessed to date.

Novel food sources in urban environments, including human-provided foods or nectar from exotic vegetation, are both abundant and predictable, and are thus expected to affect optimal foraging dynamics (17), where feeding efficiency is not only dictated by behavioral choices, but is the direct result of bill morphology (18). Many examples of rapid bill morphological change in birds have resulted from changes to resource availability, where adaptive shifts in bill shape or size permit access or increased foraging efficiency on said resource (4, 19). Bill dimensions of hummingbirds in particular are constrained by the correspondence between their bill and floral food sources (20). Specifically, longer-billed birds can reach deeper into flowers when the food source opening is larger than the bill width, but

have a disadvantage relative to short-billed birds when the opening is narrow as they make more insertion errors (20). The introduction of the hummingbird feeder may have released evolutionary constraints imposed by native flowers, as they contain nectar volumes far larger than the average flower, typically holding between 1-60 microliters of nectar (21, 22). However, feeders may also impose novel evolutionary pressures to increase feeding efficiency as they become more profitable.

We expect following the introduction of the hummingbird feeder, that foraging could be optimized by increasing the nectar volume per lick, which is achieved by making the bill longer or thicker. A length increase would accomplish getting the bill tip as close to nectar as possible, permitting an increased licking frequency and volumetric intake rate. At an artificial feeder, where nectar volume is high, there is a shorter distance between the bill tip and nectar surface. Such volumes in feeders are kept constant due to the large liquid volume, and would allow faster licking rates and thus a higher nectar extraction efficiency (21, 23). However, this may be affected by other factors such as tongue morphology or kinematics. Increased feeding efficiency can also be achieved by increasing the volume of the bill via its thickness. The tongue, which is responsible for intraoral transport of nectar, has an internal anatomy reflected by the external size of the bill (18,24). Therefore, a thicker bill is a proxy of both tongue size and internal volume for nectar-holding capacity. The consistently large volumes of nectar available via feeders are therefore expected to optimize adaptation for foraging through bill thickness and/or length.

Increases in the density of food resources often results in individuals distributing themselves according to the distribution of resources, but higher population densities can increase competition for food giving aggressive individuals an advantage (25). In AH, males engage in energetically expensive defense behavior when a large nectar source is present such that the degree of defense is a function of food quality (26, 27). Likewise, female AHs frequent *Eucalyptus* more than any other local plants, particularly when it comes to nesting and will exhibit aggression towards other nesting females (28). In lieu of such concentrated resources, bill weaponization is expected, and has been achieved many times for this reason by other species of hummingbird. North American hummingbirds of the Mellisugini clade in particular, are sexually dimorphic; thought to be the result of strong niche differences between sexes and sex-specific resource competition (29). In cases where niche-specific resources are concentrated, intrasexually selected weapons can then result from competition between members of the same sex (30). The evolution of the bill as an effective weapon would typically be relevant in the context of poking or stabbing, which is achieved by bill straightening along with a relatively wider base, in addition to a narrow bill tip, creating a higher perforation capacity and axial loading capabilities (31). Other expectations include a bill deepening to provide resistance to bending forces and the generation of conical bill tips to increase stabbing performance.

Through agonistic and foraging behavior, hummingbirds also generate large amounts of heat given their expensive flight mechanics (32), yet they retain the ability to live in hot climates (33). Particularly in birds with exaggerated bill sizes relative to body size, the bill can function to prevent hyperthermia through non-evaporative cooling at high temperatures (34). Hummingbird bills are highly vascular in nature (31) in addition to having the capacity for non-evaporative cooling (37, Don Powers Pers. Communication) suggesting they play a role in temperature regulation. Populations of other bird species occupying environments with hotter summer temperatures have increased bill sizes, while decreasing body size, relative to their counterparts living in cooler climates to promote heat dissipation (28,35). In contrast, birds that have recently colonized regions with fair summer temperatures, bill size is best predicted by critical

temperatures in winter such that bill size decreases with average winter temperatures without concurrent changes in body size (36). While other factors such as nectar viscosity in colder climates may affect the feeding mechanics of different bill sizes (38, 39), it is expected that as the AH has colonized more northerly habitat, bill size should decrease as a heat retention mechanism independent of shape.

The confluence of novel climate, food resources, and habitat are undoubtedly determining factors in the complex demographic history and recent population expansion of the AH, in stark contrast to population declines in most North American hummingbird species (37). The timing and spatial distribution of these different facets of environmental change can be leveraged to disentangle the respective effects of each on local adaptation of bill morphology. However, because bill shape and size are related to foraging efficiency, competition, and thermoregulation in different ways, changes are expected to be modular, with possible opposing selective pressures. Here, we model a panel of phenotypes related to bill shape and size over 160 years to test for temporal changes related to increasing trends of hummingbird feeder use, the introduction of *Eucalyptus*, a booming human population, microclimatic changes associated with the urban environment, and a warming climate. Here, we test the hypothesis that the introduction of high-volume nectar resources such as *Eucalyptus* and hummingbird feeders has facilitated hummingbird population and range expansion northwards into new climatic zones and that this has resulted in rapid, modular phenotypic change. Using morphometric data from over 400 museum specimens in tandem with publicly available metadata, we show that feeder density, *Eucalyptus* density, and human population density have acted together to facilitate population expansion. We also show that this expansion is associated with three facets of the Anna's Hummingbird bill morphology. Bills have increased in length and size in association with feeders and sharpened in a sex-specific manner in association with feeder or *Eucalyptus* abundance. In addition, bills have also shrunk in size as the AH colonized areas with lower average annual temperature. Our data provide evidence that the spread of urbanization can be attributed to rapid modular, morphological change and shifting evolutionary pressures on nectar feeding mechanics in a human mutualist.

Methods

Specimen Associated Sampling. Phenotypic data were measured from birds collected in California to look for spatiotemporal patterns of phenotypic change in association with urban sprawl, supplemental feeding, or climate, with respect to bill shape and size. Phenotypic data and associated metadata of AH collected over an interval of 160 years were collected from prepared skins in the Museum of Vertebrate Zoology (MVZ) at UC-Berkeley. In total, 400 specimens were located through the Arctos online database along with collection metadata to identify all specimens from California with available study skins. Metadata from Arctos included the dates collected, preparator and collector name, state and county of origin, year collected, latitude, decimal longitude, age, and weight (Figure S1). We assigned ages as either juvenile, adult, or unknown based on descriptors in the database (e.g. using phrases like 'just fledged' or 'adult plumage'), or based on bill corrugation. Unlike passerines, a double-layer of ossification does not separate juveniles from adults (41). We instead use bill corrugations, the preferred method for hummingbirds (42). Bill corrugation descriptions were classified as follows: >10% scored as juveniles, <10% or "bill smooth" scored as adults, and =10% scored as NA. Skull ossification is not an accurate descriptor of age in hummingbirds and so specimens whose age was described by

skull ossification were scored as NA (43). Missing metadata for preparators indicated that the collector and preparator were identical for that specimen (Table S1).

Each phenotype was chosen in effort to quantify traits related to body size, bill size, and bill shape as response variables for models of increased urbanization over time (Figures S2-4, Table S2). We began collecting phenotypic data for shape by taking two photographs of each specimen, one from a profile view and one from a dorsal view (Figure S5). A camera (Canon EOS Rebel T7i) was set up with a small tripod on a flat surface, with the lens about 0.3 meters away vertically from the specimen. Each specimen was photographed on a white background adjacent to a centimeter ruler and a rectangular block was placed underneath the bill to level the specimen. Flat skins were photographed with an altered procedure. For profile photos, the camera (without the tripod) was placed on the flat working surface. The specimen was laid dorsal-side up on the edge of a flat raised platform level with the lens of the camera and 30.5 cm away in distance. For dorsal photos, we used the same procedure as outlined for the study skins. However, we did not need a stand to level the specimen. Out of 400 specimens, only 386 were photographed and phenotyped as samples that were not present as skins were only used as metadata. All phenotyping was done using ImageJ on calibrated images to obtain: exposed culmen length, culmen depth, culmen width, bill curvature, body length, wing chord length, profile area, and dorsal area (44). Using a 60 mm ruler, each photo was calibrated to 20 mm.

While avian bills are expected to shrink postmortem, this typically only occurs for the first two months after death and all samples were measured outside of this interval (45). Exposed culmen length was measured from the profile view. To measure culmen length, a line was drawn from the feathers on the upper ridge of the maxilla (culmen) to the bill tip. Culmen depth was measured from the profile view where a vertical line was drawn between the upper and lower edge of the maxilla from the culmen. Culmen width was measured from the dorsal view where a line was drawn to span the base of the bill at the culmen. Bill curvature was measured from the profile view. A circle was created by placing three equidistant points along the upper ridge of the maxilla, spanning from the end of the bill feathers to the bill tip. We then obtained the radius of that circle. Curvature (C) of the culmen was calculated by $C = 1/(\text{radius})$ using methods from (46). Body length was measured from the dorsal view. A line was drawn from the bill tip to the tip of the R1 rectrix feathers. In contrast to phenotypes measured in imageJ, wing chord was measured with calipers to the nearest mm. To avoid batch effects based on preparator, all wing chord measurements for flat specimens were removed as these could not be accurately measured given the orientation of the shoulder. Body length was measured from bill tip to R1 rectrix tip. We created an additional variable describing body size by subtracting the measure for exposed culmen length from the entire body length to analyze body length changes independent of changes in bill length. Finally, we created a factor for seasonality which was generated by converting each date of capture into a continuous value representing Julian days for comparison of time of year across samples.

Traditional measurements of size and shape such as length or length-to-width ratio can be used to capture phenotypic variation, but shape is complex and may represent nonlinear functional variation. For each image, the outline of the maxilla was traced and smoothed from raw images in imageJ. The image was then cropped to include only the bill on the white background of the platform. Then, a custom script was implemented to convert the image to black and white. We converted all binary images into BMP format. Next, a chaincode was generated for all BMP files using methods from (47) and each bmp file was converted into an NEF file. For this step, the normalization method was set based on longest radius and each image

was rotated in the right window such that the tip of the bill was ordinated to the right side of the screen. A summary of shape contours were then generated using the principal components of the elliptic fourier descriptors in the NEF file (Figures 3, S4).

Database and search strategy. Alternative nectar sources, such as feeders and *Eucalyptus* plantations, may be important drivers of change in bill morphology because of their generic shape, increased volume of nectar compared to native flowers, and year-round availability. Because there are no historical records of AH feeding and *Eucalyptus* plantations that match the time scale of the collection specimens, we used newspaper mentions of hummingbird feeders and *Eucalyptus* plantations as a proxy for feeder density and *Eucalyptus* density. We searched Newspapers.com, a proprietary database containing historical local newspaper page images converted to a machine-readable format with optical character recognition (OCR). As of December 2021, the database contained 52 million pages from 542 newspapers published in California from 1852-2021. The database is not comprehensive and there are gaps in the digital archives resulting in counts of articles on any given subject that correlate with the total number of available newspaper pages. To correct for this bias, we extracted the total number of digitized pages available in each county in each year and calculated the number of pages referring to either hummingbird feeders or *Eucalyptus* plantations relative to the total number of digitized pages. We restricted our search to newspapers published between 1860-2020.

We created two sets of search terms (Table S3) to capture articles related to hummingbird feeding and *Eucalyptus* plantations, two sources of artificial or non-native nectar for hummingbirds in California. To capture references to feeding wild backyard hummingbirds prior to the commercialization of contemporary hummingbird feeders, we reviewed articles from the late 1800s and early 1900s to identify phrases used at that time to describe hummingbird feeding (e.g., using glass vials or other homemade feeders). Based on these articles, we included phrases related to hummingbirds and sugar water, however, this also retrieved articles about feeding hummingbirds as a means of taming and capturing them to keep as pets. To exclude articles that were not describing feeding of wild birds, we manually reviewed all articles retrieved by terms related to sugar water published prior to 1930, slightly more than one decade after the passage of the Migratory Bird Treaty Act that prohibits the capture and taming of wild birds. For *Eucalyptus* nectar sources, we were only interested in articles referring to *Eucalyptus* plantations, as opposed to other uses (e.g., *Eucalyptus* scents) or being tangentially mentioned (e.g., as part of wildfires) and thus we excluded terms that did not imply planting or logging *Eucalyptus*. Review of early articles indicated that references to specific types of *Eucalyptus* were nearly always referring to planting, and thus we added the terms "blue gum tree" and "red gum tree" but not "sugar gum tree" because of many irrelevant articles related to chewing gum.

The database does not allow for complex Boolean searches, however, it does allow for searching exact phrases contained within quotes and uses proximity operators for search terms not contained within quotes. Because of database constraints, we ran each line of the search strategy (Table S3) separately. This approach resulted in some articles being retrieved by multiple search terms. For example, an article containing the phrase "lure hummingbirds using a hummingbird feeder" would be retrieved by both "lure hummingbirds" and "hummingbird feeder". We exported results as a .html file and parsed the articles using a custom R script (<https://github.com/elizagrames/altNectar>) to obtain the newspaper name, date and location of publication, and page number for each article. Article titles and content were not exported due to

database constraints. To remove duplicates, we retained only unique records based on the newspaper name, date, location, and page number.

Human Population Density. Housing density or urbanization of the landscape *sensu lato* are known to be important for the reduction in migratory behavior, frequency of overwintering, and density of feeders (5). We used human population density as a proxy for “urbanization” or housing density. Metadata for human population density came from the United States Census Bureau website where the decennial census numbers were taken from each county in California back to 1860 (Figure S7). To predict population sizes in interim years, a linear model was fit between the year and log population size of each county. We used the predicted log population size with an exponential transformation to fill in missing data.

Feeder and Eucalyptus Density. We modeled feeder and *Eucalyptus* density as putative drivers of AH density and phenotypic change given the widespread distribution of supplemental feeding and links to the range expansion (5). Absolute or estimated counts do not exist for either parameter by year or county, so we estimated densities through word counts as a proxy for usage. We took the data from the string-matching step and made the assumption that density increased monotonically and only increases from the previous maximum value. Each interim year containing a “false” zero was given the previous maximum value of “feeder” counts. We then perform a linear regression on the log transformed value over time for each county and exponentially transform the predicted value for interim years (Table S4, Figures S7-8).

Hummingbird Population Density. AH population density was estimated to model population size as a function of *Eucalyptus*, feeder, and human population density to predict which of these may or may not be important drivers of range expansion and local population size. Local population density was assessed by identifying the nearest Christmas Bird Count (CBC) circle to each collecting locality and calculating the number of AH reports per party-hour (following (48) and (49)). We then plotted a loess curve of bird density over time using ggplot2 in RStudio (Figure S6)(49).

Climate Data. We tested the hypothesis that bill size should track mean temperature as the AH expanded its range into new climatic zones. We extracted average monthly temperature, maximum monthly temperature, and total precipitation for the time and location of each specimen collection from the PRISM dataset of interpolated historic climate data (50) at 4 km resolution.

Thermal Imaging. Videos were taken with a FLIR thermal camera. Radiometric JPEG images are then processed through R (using the software Exiftools and the R package ThermImage) to assign a temperature value to individual pixels (Figure 4). These RAW data files are then processed through ImageJ. Specific areas of interest are outlined, with the mean values of these shapes correspond to the mean temperature of the given area.

Linear Mixed Models. Each phenotype was modeled as a response variable to test for temporal changes related to various types of environmental change. Specifically, each phenotype was modeled as a response variable with human population density, feeder density, *Eucalyptus* density, or climate across time as explanatory variables. In this case, the majority of the samples

were collected from a subset of counties and prepared by a subset of individuals. Because our data had uneven sampling such that few levels had most of the data with respect to county and preparator, these variables could not be incorporated as fixed predictors and were designated as random effects. We were additionally unable to incorporate these factors as random effects in a frequentist framework given that some levels had a single value and instead used a Bayesian MCMC approach using MCMCglmm v.2.3 in R v.4.1.1, incorporating both preparator and county as random effects (51, 52). Age was not estimable in all samples (See Specimen Associated Sampling), and was incorporated as a fixed effect, removing all samples with unknown age from analysis.

We included the independent variables year, sex, and age with county as a random effect in all models. Covariates for models were decided based on correlation between phenotypic variables (Figure S12). Abiotic covariates were highly correlated with one another (feeder density, Eucalyptus density, and population density), so we implemented a separate model for each (Figure S13). We additionally ran a base model without covariates besides age and sex as a positive control for temporal effects. Finally, we incorporated a climate model that included mean annual temperature and mean monthly precipitation as covariates. Climate was modeled separately as these data were only available for samples collected after 1895. Thus, for each phenotype of interest, we ran five different linear mixed model frameworks: a population model including the effects of population and an interaction between population and sex, a feeder model including the effects of feeder density and an interaction with sex, a Eucalyptus model including the effects of Eucalyptus density and an interaction with sex, a climate model including the effects of mean temperature and mean precipitation at the sampling locality, and a base model including the effects of year, age, sex, and county as random effects (Tables S5-18). The three exceptions to this were: 1. the models of bird population density, in which a standard linear model was used and included year, latitude, feeder density, human population density, *Eucalyptus* density, and feeder density by year as covariates (Table S19), 2. Models of body length, which also included preparator as a random effect. Because body length was measured from bill tip to tail tip, we first converted this measurement into a body size only measure to exclude the effects of bill length changes independent from body size. This was accomplished by subtracting the measured value for exposed culmen from body length into a new measure, and 3. We included Julian day of the season as a covariate in models of weight as we expect fat content to vary with season.

All response variables were normalized using the mutate function in dplyr v.1.0.7 to permit downstream comparison of effect sizes between phenotypes (53). Before running each model, the dataframe was filtered to remove NA's for each component in the model using the filter_at function in the dplyr package. All models used weakly informative priors using the following parameters: $V=1$, $\nu=0.002$, $\alpha_{\mu}=0$, and $\alpha.V = 25^2$. When running MCMCglmm, we set the number of MCMC iterations for each model to 65,000 iterations with a burnin of 15,000 iterations and a thinning interval of 50, which was sufficient to reach convergence in all models as determined using trace plots.

For the first two principal components of dorsal and profile shape, we ran an additional linear model using base R to determine the interaction of latitude, time, and feeder or Eucalyptus density on bill phenotype (Table S20). This allowed us to produce a spatiotemporal model for how these changes proceeded. We did not include any random effects such as county as we use latitude to incorporate spatial information. We first transformed all four principal components to be normally distributed using an arcsine transformation in R. For profile principal components 1

and 2, we included year, sex, and age in addition to feeder density, latitude, and interaction effects of both on year. For dorsal PC1, which had a significant interaction effect between sex and feeder density, we instead included sex as an interaction term for all covariates besides year, sex, age, and latitude. For dorsal PC2, we previously found a significant effect of *Eucalyptus* density and an interaction effect of sex and *Eucalyptus* density. In this instance, we included year, sex, age, *Eucalyptus* density, latitude, and the following interaction terms: sex:density, sex:density:year, and sex:density:latitude. We then implemented the step function for all models to iteratively drop terms until the best model is determined. All resulting significant interaction trends were determined using xyplots (54).

Results

Over the course of the 20th century, human populations and feeder density increased in California from South to North with localized increases in *Eucalyptus* density. Predicted annual human population sizes by county show population size increasing first and most rapidly in southern California in the 1920's (Figure S7). It is not until the 60's and 70's, that population size begins to increase in Northern California. Counts of terms associated with hummingbird feeders, as a proxy of density, increased rapidly across counties from south to north during this time, shortly after the population begins to grow rapidly in southern California. Specifically, counts in Los Angeles began as early as the 1930's, whereas other southern counties don't show increases until the 50's. Counties in northern California then began to increase feeder-related term usage in the 60s-70's, specifically in Sacramento and Stanislaus counties around 1970 (Figures S8, S9). *Eucalyptus* terms, in contrast, increased in density more gradually and over a longer period of time from the early 1870's (Figure S10, S11). While *Eucalyptus* terms increased most rapidly in Los Angeles County in 1870, terms increased gradually and locally shortly after this time in counties across the state.

Anna's Hummingbird population sizes show similar patterns to human and feeder density whereby bird populations increase rapidly over time with all measured aspects of urbanization: human population size, feeder density, and *Eucalyptus* density. Bird population density as measured by birds per hour in feeder abundance models showed a positive association with feeder density and year where bird population density increased more strongly at southern relative to northern latitudes over time (Table S19, Figure S6, S14). When survey location was not included as a random effect, instead relying on latitude for spatial information, there is a positive association with all terms: human population, feeder abundance, *Eucalyptus* abundance, latitude, and latitude by feeder abundance (Table S20).

The shape of AH bills in association with feeders elongate, deepen, and form a bulge at the bill tip as the density of feeders increases, subsequently referred to as "feeder bills" (Figure 3; Tables S5, S10-13). Culmen length increased locally with human population size and supplemental feeding in both sexes in association with feeders (Table S5). Concurrent with changes in culmen length in association with feeders, the bill deepened and narrowed in width for both sexes. Despite bill deepening at the base, profile area decreased in association with population size and supplemental feeding while that of dorsal area increased in association with supplemental feeding (Tables S10, S13). A shift in profile shape towards the feeder bill shape (lower values of profile PC1) are explained uniquely by feeder density corresponding to a decrease in the ratio of bill depth to length with a bulge at the bill tip (lower values of profile PC1), and a taper towards the edge of the bill (higher values of profile PC2) (Tables S5, S7, S11-

12). Both shape trends represent 68.9% and 13.4% of the variance in profile shape, respectively. Bill length was positively associated with the density of feeders indicating that feeder bills are longer (Figure 1). Feeder bills are both deeper at the base and have a larger dorsal area. Because bill depth increased and profile area decreased in both sexes in association with supplemental feeding or human population size, and feeder bills have a lower depth to length ratio, feeder bills are overall larger, at least outside of constricted regions of the bill center (Figures 1, S19; Tables S7, S13).

Shifts in dorsal shape are sex-specific with female-specific associations with *Eucalyptus* density and male-specific effects with supplementary feeding and housing, in both cases generating sharper bills (Figure 1). Sex-specific effects of bill shortening and acumination in males is associated with supplemental feeding/ human population and are referred to as “feeder males” (Table S5). This association with feeder density in males corresponds to bills with a higher width to length ratios along the length of the bill, resulting in increased sharpness (Figure S5, Table S14). Male birds additionally exhibit decreased wing chord length in association with higher human population sizes (Table S17). In contrast to male-specific changes in association with feeder density, female-specific changes are associated with *Eucalyptus* density and are characterized by a strong tapering from a dorsal perspective, increasing bill sharpness and are referred to as “*Eucalyptus* females” (Figure 1; Table S15). These female-specific shifts in dorsal shape occur without distinct changes in dimensions of size, instead displaying a less uniform width and stronger tapering along the length of the bill.

Lastly, models of bill shape change in response to climate associate shorter bills and smaller dorsal area with colder mean temperatures (Figure 4; Tables S15, S13, S21). We found that culmen length is positively associated with mean temperature and marginally with monthly precipitation (Table S5), while width is only marginally associated with mean temperature (Table S6). Additionally, dorsal area is positively associated with mean annual temperature and monthly precipitation (Table S13). In contrast, depth is negatively associated with mean temperature (Table S7). A consequence of these trends is that there is a gradual increase in both dorsal area and culmen length with iterative increments of 5 degrees Celsius (Figure 3). The hummingbird bill can be modeled as a cone, where the combination of decreased depth, increasing dorsal area, and increasing length imply a positive association between bill surface area and mean annual temperature. There were no associations between any of the three aspects of body size with climate or time of season.

Discussion

Manifold changes to the Anna’s Hummingbird’s environment have included a changing climate in addition to increased urban sprawl over the past 200 years. The scale of these changes have resulted in local declines of species on a global scale, particularly with respect to birds, which experienced a cumulative loss of around three billion birds in North America in the last 50 years, including hummingbirds (45, 55). Despite patterns of population decline in hummingbirds across North America, the AH has thrived, having increased in size since the early 1900’s and more dramatically following the 1970’s (37, 49). Here, we link increased population size densities of the AH to many independent factors, supporting the idea that the confluence of warming winter temperatures, urbanization, supplemental feeding, and introduced plants likely facilitated a range and population expansion in a non-uniform way (5, 49). Population density decreases with latitude over time as the AH has moved northwards, and is

consistent with supplemental feeding as a driver given that bird population density increases concomitantly with feeder density from south to north. In tandem with this expansion, we show modular changes in beak size and shape associated with thier drivers using museum specimens from 1860-present (Figure 1).

Bill Size and Shape is Associated with Supplemental Feeding

Supplemental feeding of wild birds is popular world-wide, with around half of North American households participating in this pastime (56). In regions where bird feeding is widespread, heritable changes in bill morphology have suggested that longer billed birds have a higher propensity to use seed feeders and have higher fitness than their counterparts where feeders are common (4). However, the bill morphology of nectar-feeding birds, have not been examined as a consequence of supplemental feeder introductions. Although it likely existed earlier, we report that the widespread pastime of feeding hummingbirds can be traced back to an article published in National Geographic in 1928 documenting how to ‘tame’ hummingbirds by making bottles of sweet liquid masquerading as flowers (57), and is thought to have directly influenced the first patented hummingbird feeder in 1947 (58). As a result of this newly popularized feeder, instances of terms associated with hummingbird feeders increase rapidly from southern to northern California where feeder density began its increase in the historic range and followed AH populations as they moved north (Figure 2).

Hummingbirds represent a highly specialized group adapted to nectar-feeding, where optimal foraging theory predicts the benefits of shape and size matching between bills and nectar sources (59). The feeding efficiency of hummingbird bills with respect to bill morphology has been well studied, where long vs. short bills offer distinct advantages and costs at nectar sources (20). The bills of the AH increase in length in association with feeder density, indicating that these birds exhibit a larger maximum extraction depth. This was concomitant with increases in bill depth and overall bill size, providing both the advantage of increase internal volume in addition to decreased distance to the nectar surface with bill elongation. Two distinct aspects of shape occur with these changes, each with their own advantages. The first axis of variation in profile shape describes a distinct shift from a cone-like profile to one with a constriction near the bill center and a bulge at the bill tip (Figure 3). This shape is consistent with an exaggeration of internal structures, including a bill wringer and four nectar basins near the bill tip known to increase efficiency of nectar offloading (60). The second axis of variation in profile shape indicated that bills have shifted to a more tapered shape whereby the depth decreases more as you move away from the base and may facilitate deeper extraction depths or perforation capacity. However, while the hummingbird bill is a nectar extraction tool, males and females also compete for territory and resources, creating additional selective pressures and constraints that come from using the beak as a weapon (31).

Bill Sharpness is Linked to Resource Density in Males and Females

Male AHs will defend exclusive territories with energetically expensive behavior when food is concentrated and abundant as is the case with supplemental feeders (26). Because the availability of nectar is directly related to territorial defense, which is itself related to feeding efficiency, sex specific changes are expected with respect to food resource availability (61). Likewise, female AHs use *Eucalyptus* more than any other local plants, particularly when it comes to nesting and will exhibit aggression towards other nesting females (28). 20th century naturalists noted that introduced *Eucalyptus* was a likely source for the increase in carrying

capacity of the AH population as trees were abundant and the flowering season overlapped with the AH breeding season (49). Morphological changes to the male and female bill in response to feeder and *Eucalyptus* density, respectively, become evident in AH when viewing the bill from a dorsal perspective (Figure 1). In the case of males, feeder and human population density is associated with more tapered bills. This change, in effect, produces a sharper bill which should provide higher perforation capacity and axial loading to increase stabbing performance and resist perpendicular bending (31). From a female's perspective, the density of *Eucalyptus* is associated with a shift towards a sharper bill. In both cases, bill width is relatively constant over time, indicating sharpness is achieved by bill width decreasing as one moves towards the bill tip. Females additionally use their bills in the capture of insects, nest building, and feeding offspring suggesting that female-specific shifts may have additional functions, such as increased access to *Eucalyptus* flowers. However, *Eucalyptus* flowers have large, open flower structures, and hummingbirds typically eat insects with the gape of their bill, achieving this with increased jaw flexibility. Additionally, while this sexual dimorphism may be related to nest building, nest structure is conserved in many hummingbirds while bill shape varies widely between species. Other forces such climate are also known to constrain bill morphology in that some birds regulate body temperature in hot climates by dissipating heat from the bill surface (62).

Bill Surface Area is a Thermoregulatory Adaptation to Climate

Hummingbirds have the ability to live in hot climates, yet generate large amounts of heat as the power required for hovering flight is high (63). Particularly for birds with energetically demanding flight like hummingbirds, a large bill would help dissipate heat to prevent metabolic overheating (64). The high degree of vasculature in bird bills, including hummingbirds, make them poor insulators. However, in birds occupying warm environments, these are additionally utilized as efficient thermoregulatory organs that regulate heat distribution in the body (34).

The climatically variable habitat of the AH ranges from hot dry summers in the historic range to cold winters and mild summers in their expanded range. In some bird species with similarly variable ranges, bill size is correlated with average air temperature, such that a higher bill surface area better dissipates heat in hot climates and retains heat in cold climates (36). Here we show that bill size and area are strongly associated with mean annual temperature such that bills shrink in size with increasing latitude, which would be expected if the hummingbird bill functions as a thermoregulator (Figure 4). We find support for this with thermal imaging data to show that nonevaporative heat dissipation occurs during perching, suggesting this pattern likely represents a heat retention mechanism in these novel environments. However, other explanations for bill length decreases may include variation in the nectar viscosity in different temperature regimes, which ultimately would alter feeding mechanics. We propose that as hummingbirds have expanded their range to the north, the reduction in bill size was a consequence of local adaptation to colder winter temperature (36), despite having the opposite effect in association with increased local feeder densities. We had the unique ability to disentangle these opposing effects due to the highly localized nature of temperature and resource abundance, where relative effects of environmental changes on phenotypes could be measured.

Concluding Remarks

The expansion of the AH is linked to the confluence of multiple overlapping changes to the environment including a growing human population, the introduction of non-native *Eucalyptus*, and the popularity of supplemental feeding. This presents a complex model of

population expansion with simultaneous shifts in morphology on different axes related to temperature regulation, optimal foraging, and conspecific competition. As human populations begin growing northwards, so too are hummingbird populations, which begin growing since the early 1900's (49). Nonnative plants such as *Eucalyptus* were likely involved in the initial increase in hummingbird population size and range as feeders had not gained popularity at the time. It is then likely feeders which facilitated the following exponential population increase northwards in the late 20th century, where birds have a greater reliance on feeders relative to their southern counterparts (5, 49). The movement of hummingbird populations northward then exposed them to new climatic conditions which may have imposed physiological constraints on heat conservation during the cold winters. Here, we have shown that bill size and profile shape are linked to supplemental feeding, with known consequences for foraging efficiency. Males, in association with human population density and feeders, and females, in association with *Eucalyptus*, experience supplementary shifts in beak sharpness likely as a result of their differentiated ecology. We show that the bill functions as a thermoregulatory organ when perching and that this has driven subsequent bill size decreases independently in association with lower temperatures in the expanded range. Our study illustrates the importance of metadata and museum specimens in predicting species trajectories, particularly in association with growing human populations. On the other hand, we have shown that responses to environmental change can be rapid, and modular in the face of many, often opposing, forces.

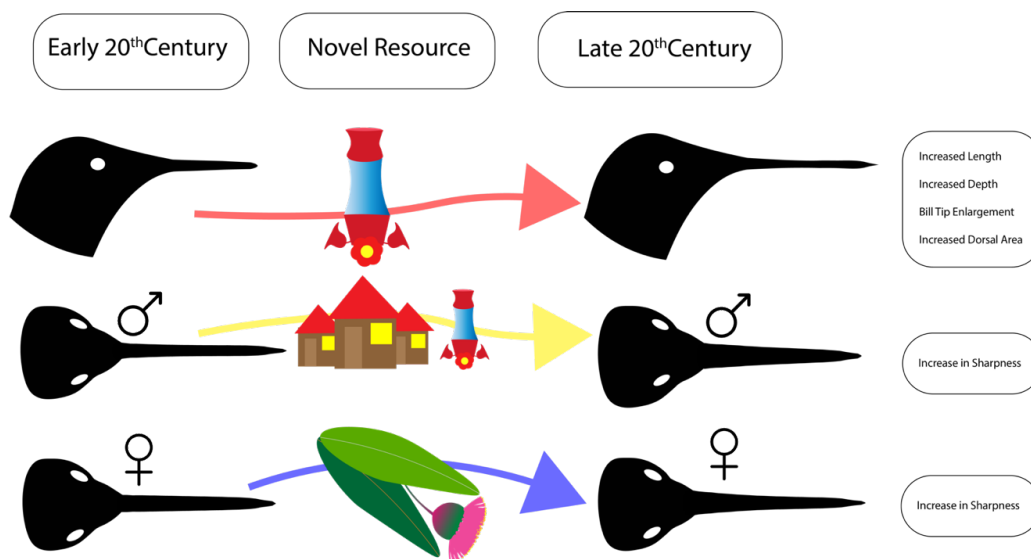


Figure 1. Graphical summary of results for bill morphology changes in association with resource density. Feeder density is associated with both bill elongation and deepening in addition to distinct bill enlargement at the bill tip. Bill sharpening is resource and sex dependent such that males exhibit bill sharpening in response to human population density and feeder density, and females linked to *Eucalyptus* density.

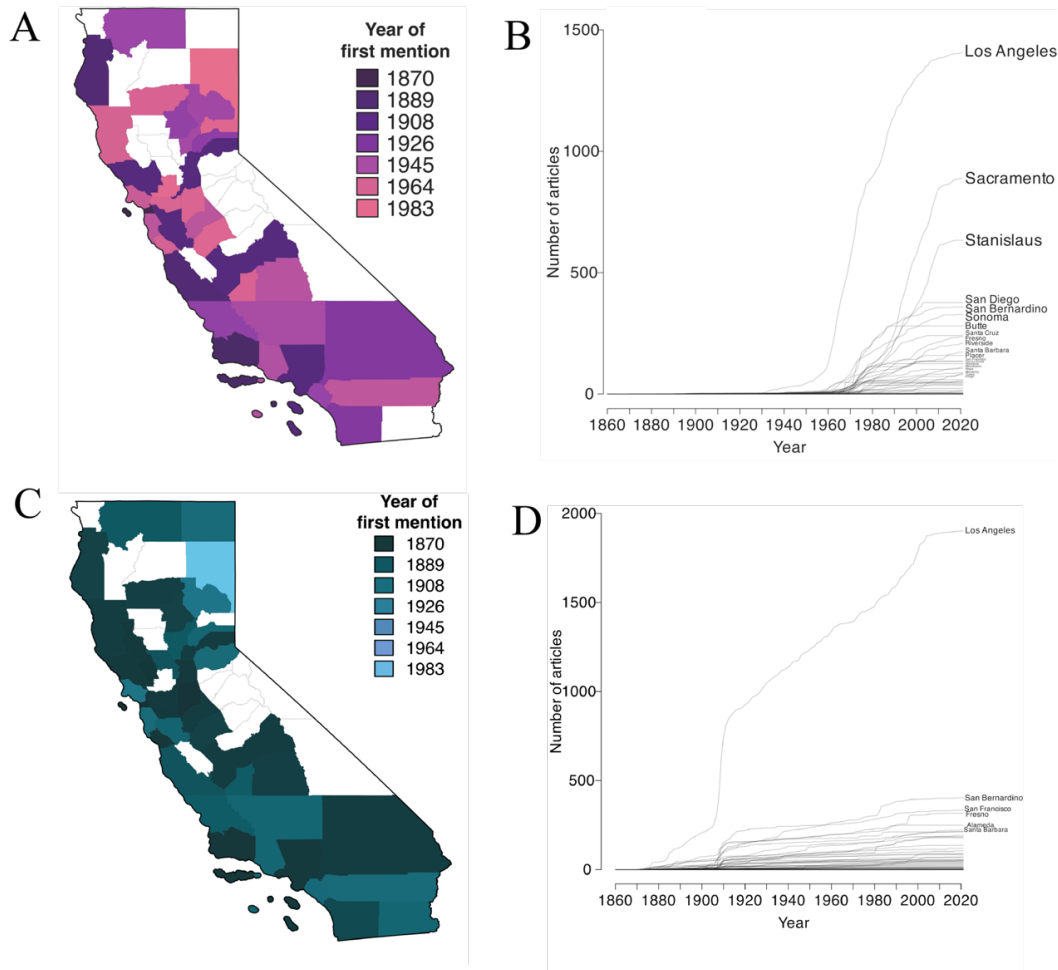


Figure 2. Spatial distribution of search term counts for supplemental feeders and *Eucalyptus* by county. All counts are based on the number of articles mentioning relevant search terms in each county in each year (See Table S3). A. Year of first mention for feeder-related terms. B. Number of articles with mentions of feeder-related terms over time by county. C. Year of first mention for *Eucalyptus*-related terms. D. Number of articles with mentions of *Eucalyptus*-related terms over time by county. Search term density increases for hummingbird feeders in southern counties first with gradual increases at northern counties over time, matching expectations based on human population size trends. *Eucalyptus* mentions begin early with gradual increases that are localized without a latitudinal trend.

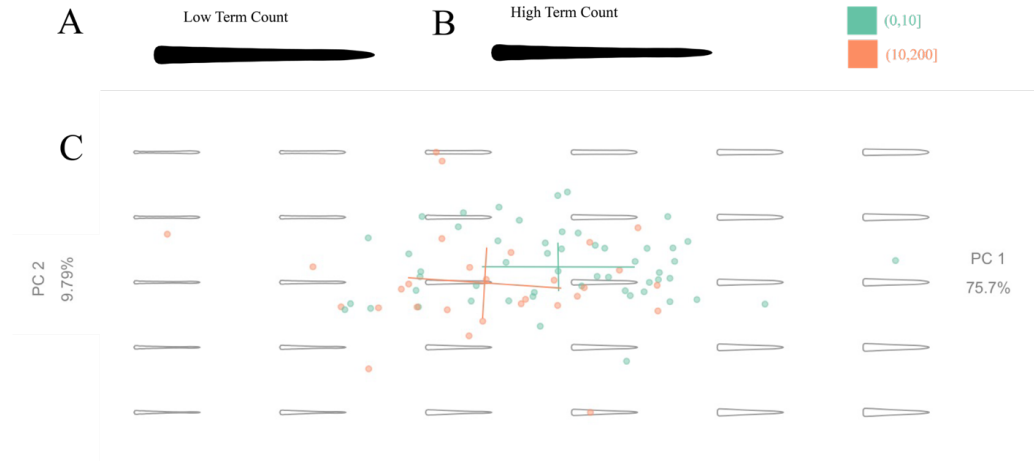


Figure 3. Summary of localized trends in mean profile shape shifts in association with feeder density. A-B. Average profile contour for all adults in Alameda county from the year 1940 or above with data binned into counts of feeder-related term mentions between 0-10 and 11-200. C. Panel C shows the decrease in profile PC1 in association with feeder density increases. Principal components 1 (y-axis) and 2 (x-axis) of profile shape plotted in 2D-space. All points represent adults from above 37 degrees latitude north. This filter was chosen for display purposes as birds at higher latitudes are more reliant on feeders, although the same trend holds without the latitude filter. Birds in regions of higher feeder density have lower values of profile PC1 and higher values of profile PC2 resulting in a tapered bill with a lower depth to length ratio.

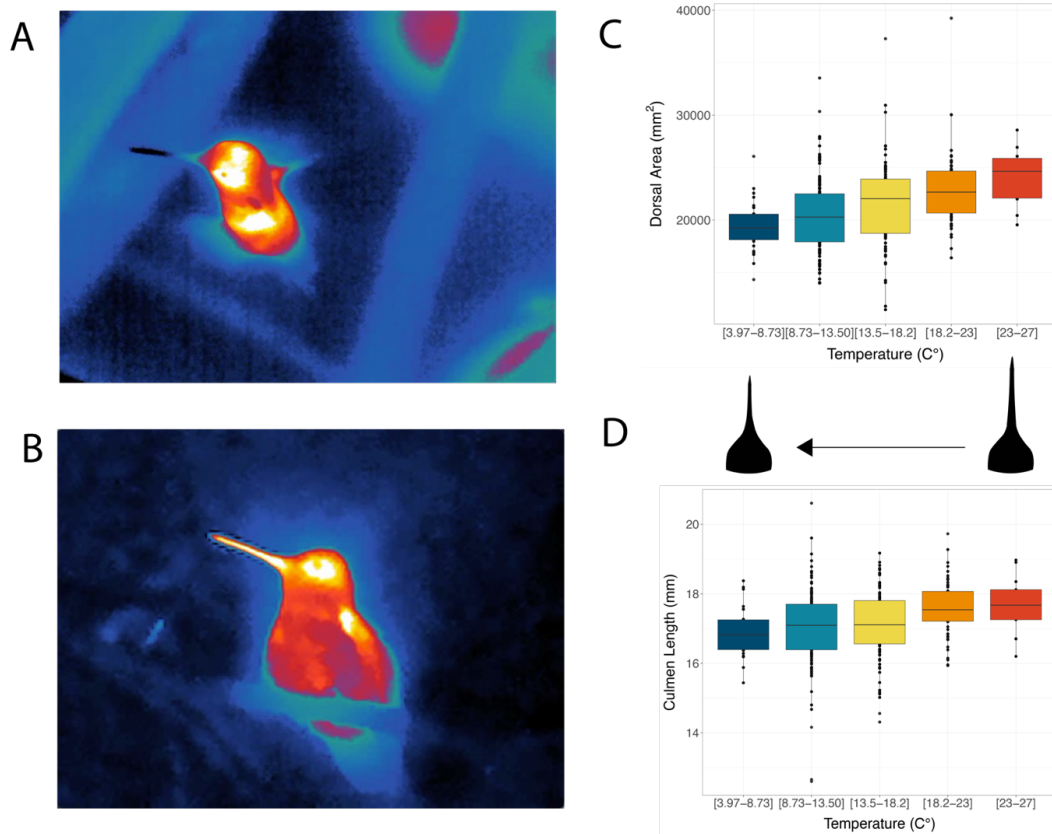


Figure 4. Screen captures of thermal videos of individual Anna's Hummingbirds exhibiting different locomotory behaviors A. Anna's Hummingbird in flight displaying heat is primarily dissipated from the eyes and feet. B. Thermal imaging suggests the bill dissipates heat primarily during perching. Colors in the red-orange color range indicate warmer surface temperatures, while colors in the blue-purple color range indicate cooler surface temperatures. C-D. Dorsal area [C] and exposed culmen length [D] as a function of mean annual temperature. Mean annual temperature of the collection locality for each specimen was placed into 5 equally spaced bins of mean annual temperature and plotted against the dorsal area or culmen length. Dorsal area and culmen length are positively associated with mean annual temperature.

Supplementary Figures

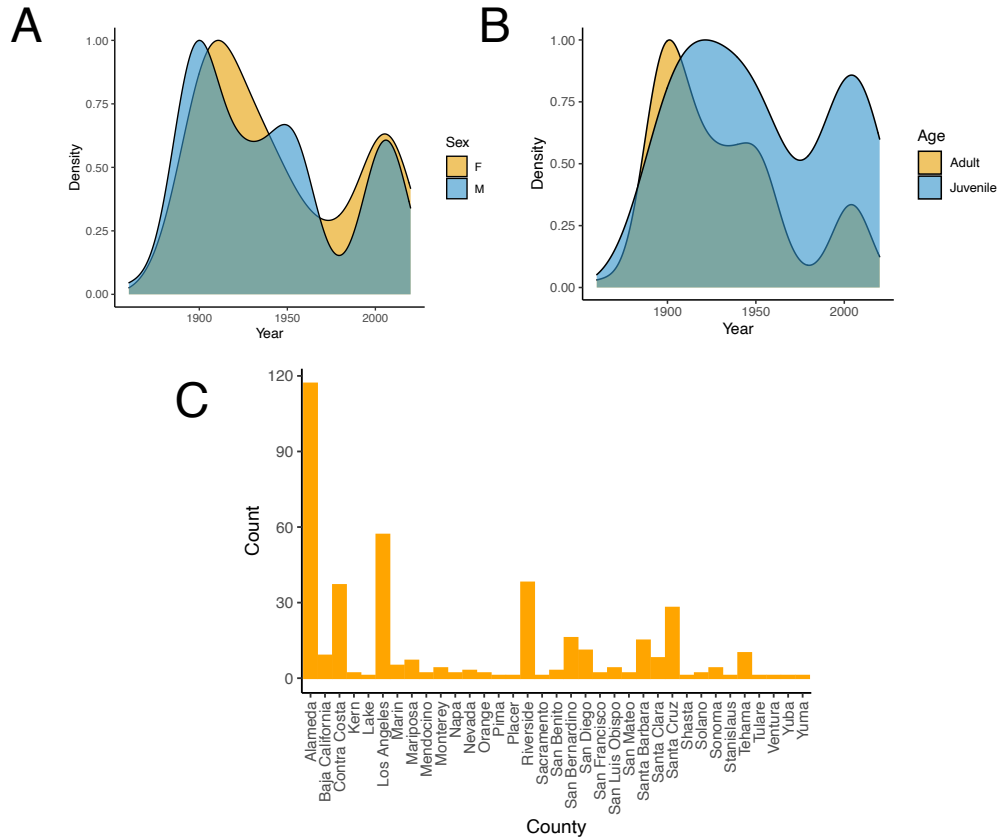


Figure S1. Distribution of data by level for fixed and random effects. A. Density plot depicting relative number of samples belonging to each category of sex across the sampling interval. B. Density plot depicting relative number of samples belonging to each category of age across the sampling interval. For both density plots, the area under the curve between each set of x-values represents the probability of sampling from within that interval relative to the whole set. C. Histogram showing the count of samples for each county sampled.

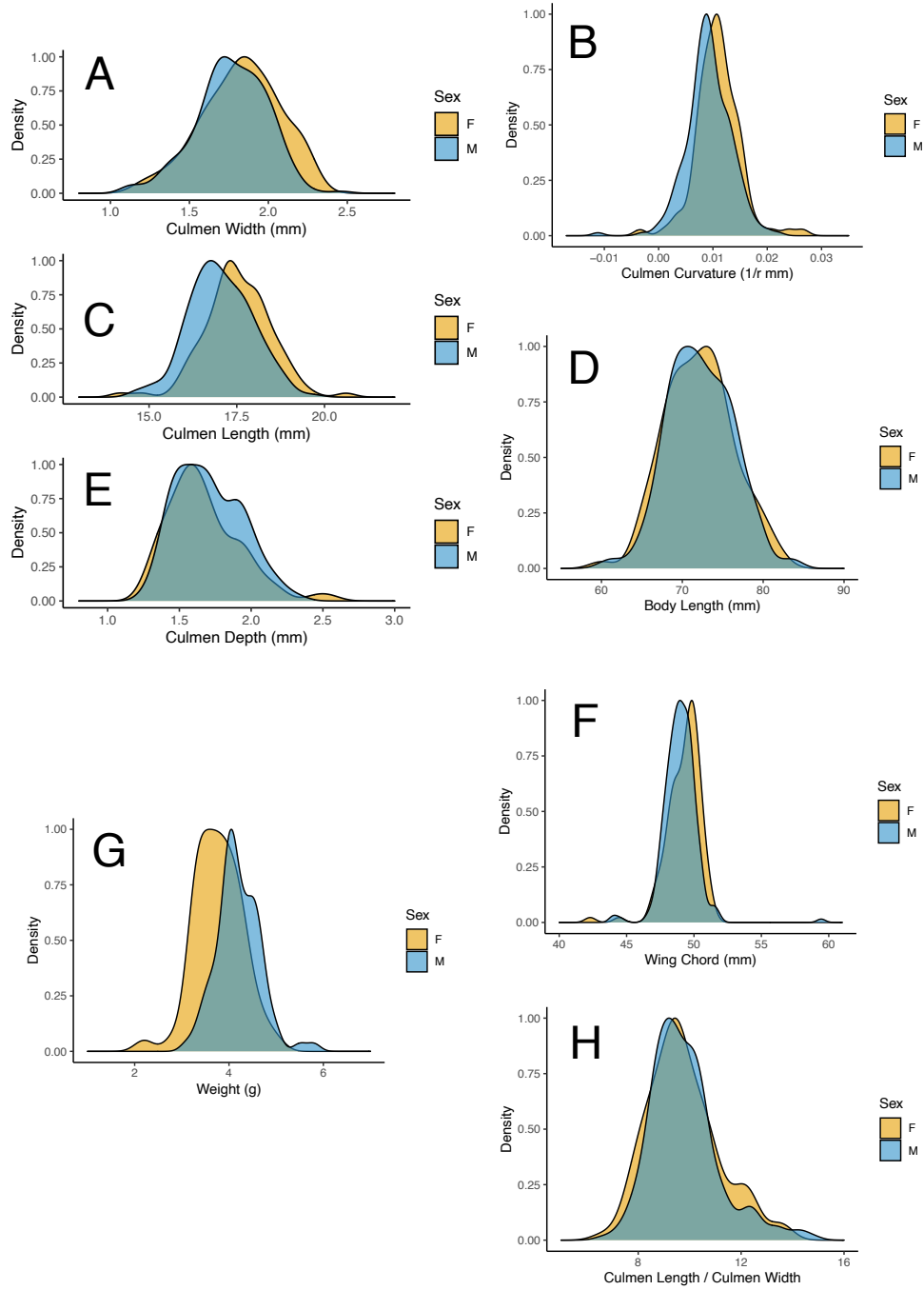


Figure S2. Density plots depicting relative number of samples within each range of phenotypes by sex. For all density plots, the area under the curve between each set of x-values represents the probability of sampling a phenotypic value within that interval relative to the whole set. A. Density plot of culmen width measured in millimeters. B. Density plot of culmen curvature measured as 1 divided by the radius of a circle fitted to the maxilla surface with three equidistant points. C. Density plot of culmen length measured in millimeters. D. Density plot of body length measured in millimeters. Body length was measured as the distance from the tip of the maxilla to the R1 rectrix feather. Exposed culmen length was then subtracted from this value. E. Density plot of culmen depth measured in millimeters. F. Density plot of wing chord measured in millimeters. Wing chord was measured as the Euclidean distance from the shoulder to the wingtip. G. Density plot of weight at capture measured in grams. H. Density plot of the ratio of culmen length measured in millimeters over culmen width measured in millimeters.

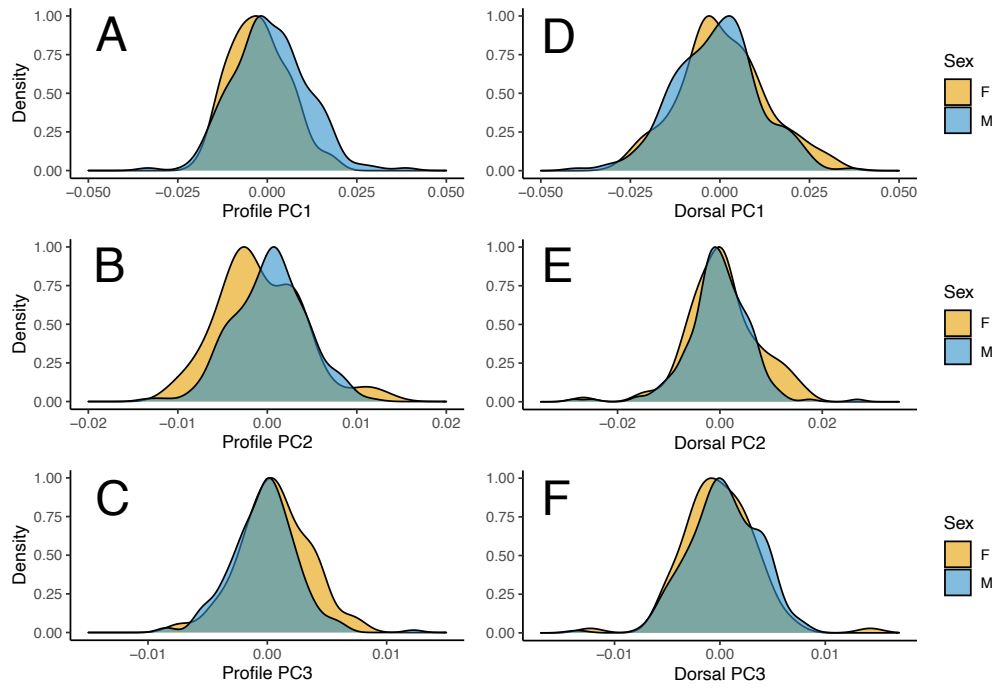


Figure S3. Density plots depicting relative number of samples within each range of principal component values by sex. For all density plots, the area under the curve between each set of x-values represents the probability of sampling a principal component value within that interval relative to the whole set. All principal components were centered and scaled. A. Density plot of profile principal component 1. B. Density plot of profile principal component 2. C. Density plot of profile principal component 3. D. Density plot of dorsal principal component 1. E. Density plot of dorsal principal component 2. F. Density plot of dorsal principal component 3.

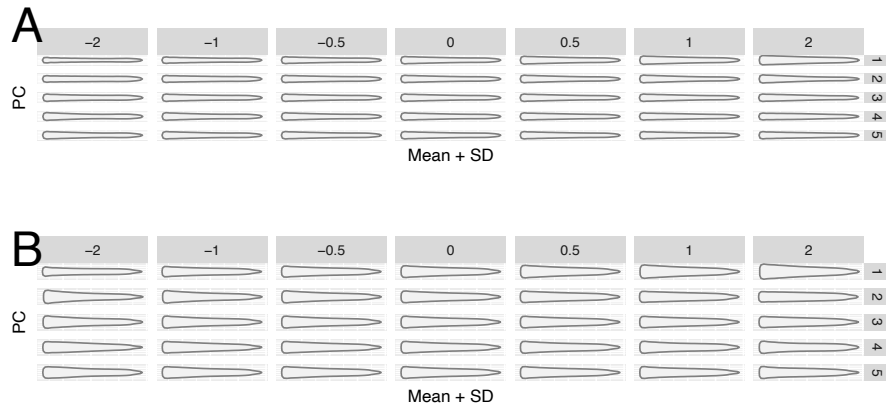


Figure S4. Mean centered principal components of bill shape for profile shape (A) and dorsal shape (B). Variance around the mean, centered at zero, is displayed as two standard deviations on each tail of the mean. The first to fifth row of each grid represents the first to fifth principal components, respectively. The above images are based on contour shapes traced in imageJ and converted into a bitmap image. The contour shape is then extracted from the bitmap image (BMP file) and delineated with elliptic fourier descriptors (EFD) (47). A principal component analysis was then performed on these EFD's.

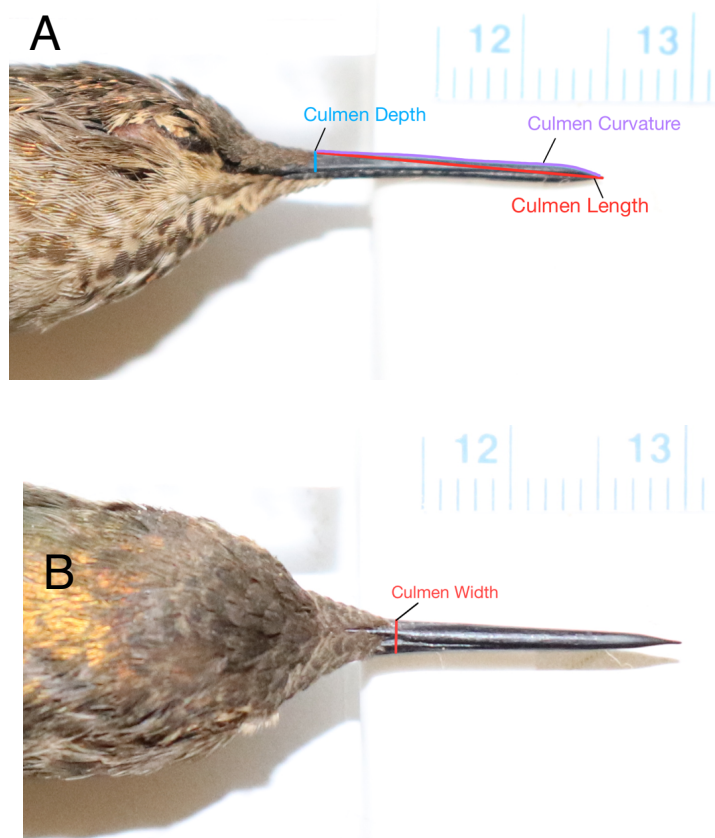


Figure S5. Photograph of an individual from a profile (A) and dorsal image (B). The culmen refers to the point on the maxilla where the feathers begin. A. Culmen Depth and Length were always measured from profile images. Culmen depth refers to the depth of the maxilla measured in millimeters. Culmen length refers to the depth of the maxilla measured in millimeters. Culmen curvature was determined using a three point ROI. Three points were placed equidistantly along the maxilla to generate a circle. The inverse of the radius was used as a measure of curvature. B. Culmen width was always measured from dorsal images and represents the width of the bill measured in millimeters at the culmen.

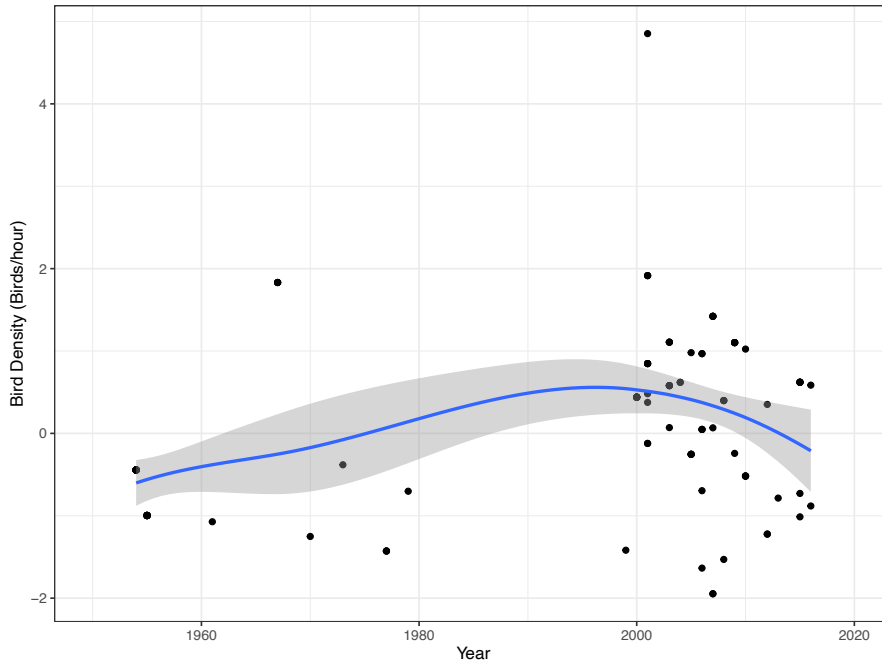


Figure S6. Loess curve displaying birds per hour as a proxy for bird density data between ~1940-2015. Data for the state of California suggest that bird density has increased over time. These increases are associated with multiple changes to the environment over the course of the 20th century including human population density, supplemental feeder density, and *Eucalyptus* density.

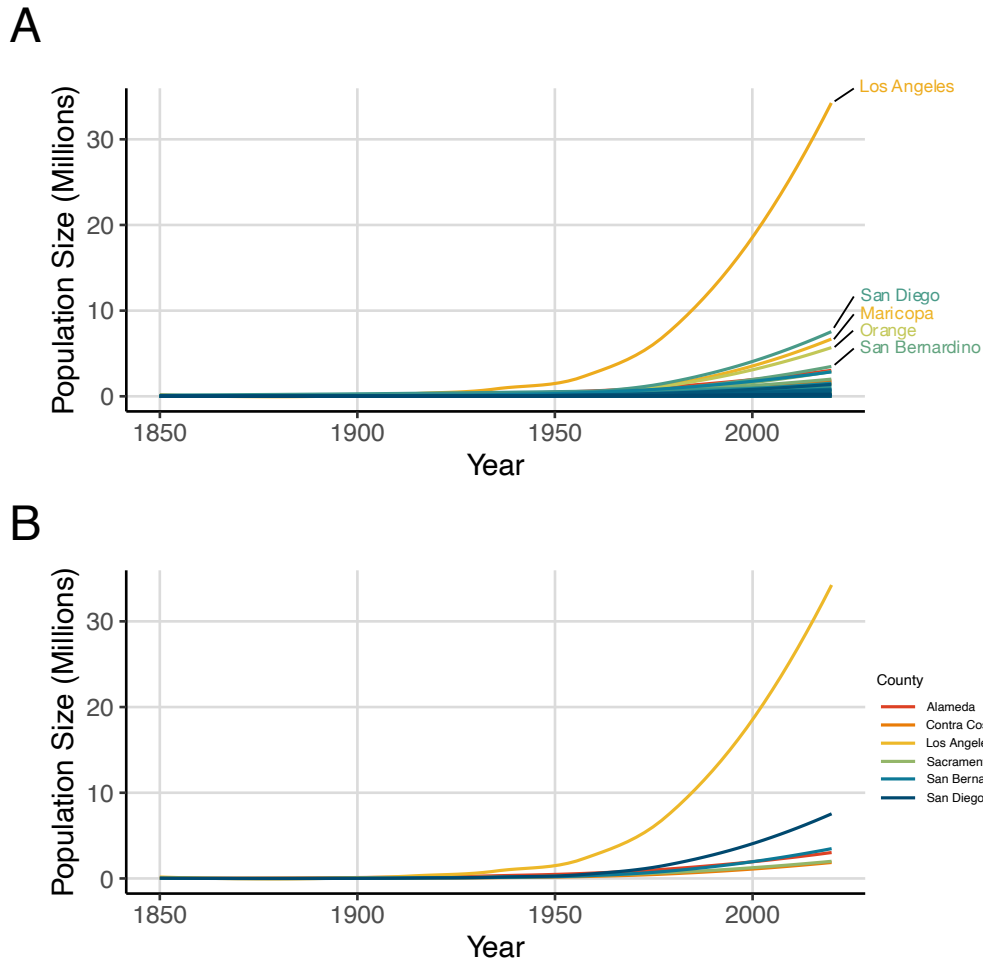
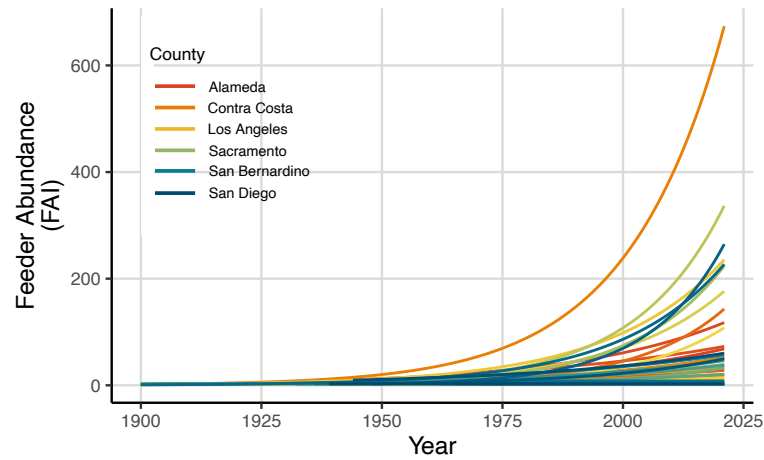


Figure S7. Human population size over time by county. The population size of each county in each year was determined using decennial census data for each county in California. Counts for interim years were predicted using exponentially transformed predictions of a linear model of the log population size over time fit to the data. The above figures show human population size as a function of year for all counties (A) and for six relevant counties (B). Human population size increases over time in California, with exponential growth beginning in southern California and later extending to more northern counties.

A



B

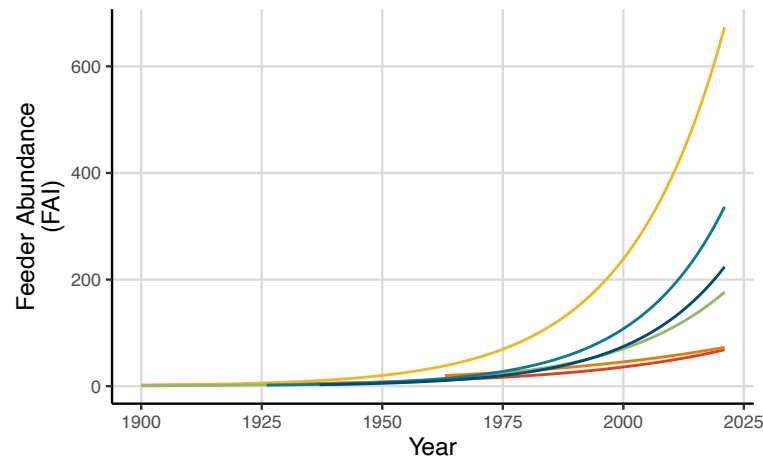


Figure S8. Predicted feeder abundance over time by county. Feeder abundance of each county in each year was estimated using the number of articles mentioning relevant search terms in each county in each year (See Table S3). Counts for interim years were predicted using exponentially transformed predictions of a linear model of the log number of articles by year fit to the data. The above figures show predicted count of articles as a function of year for all counties (A) and for six relevant counties (B). Feeder density increases exponentially over time in California, with exponential growth beginning in southern California and later extending to more northern counties.

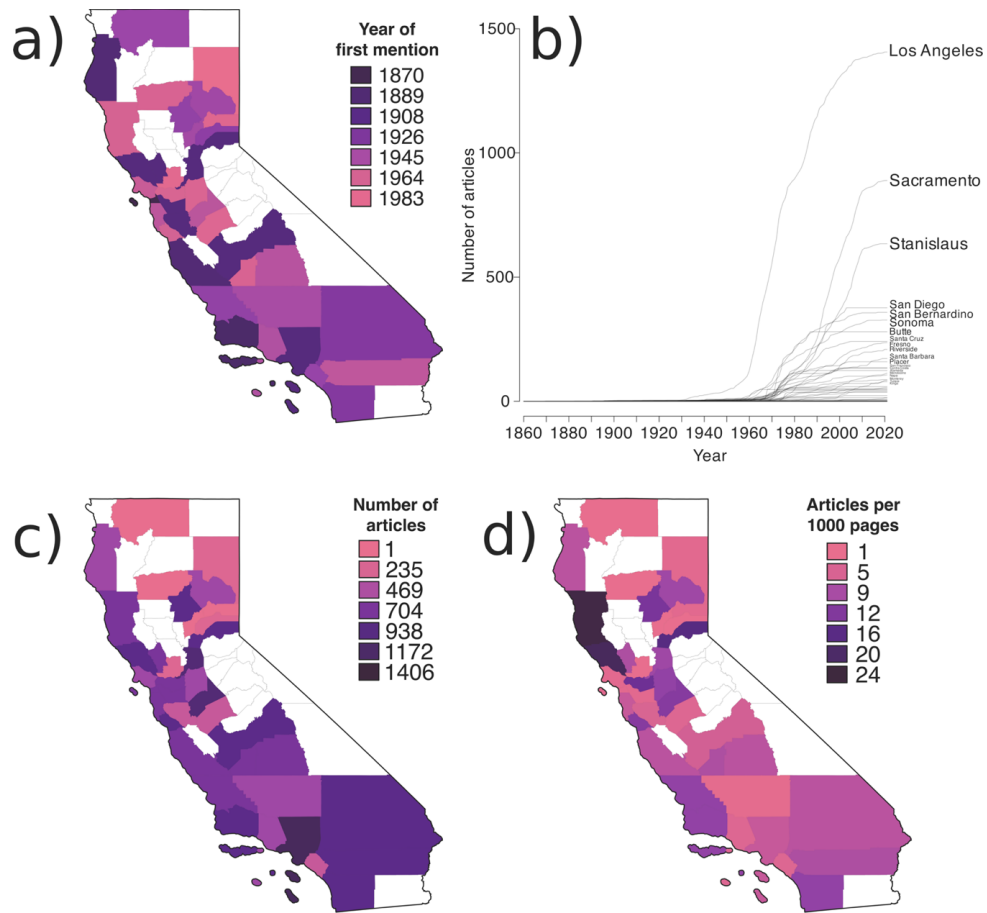


Figure S9. Spatial distribution of search term counts by county. All counts are based on the number of articles mentioning relevant search terms in each county in each year (See Table S3). A. Year of first mention for feeder-related terms. B. Number of articles with mentions over time by county. C. Number of articles with mentions by county. D. Number of articles with mentions per 1000 pages. Search term density increases in southern counties first with gradual increases at northern counties over time, matching expectations based on human population size trends.

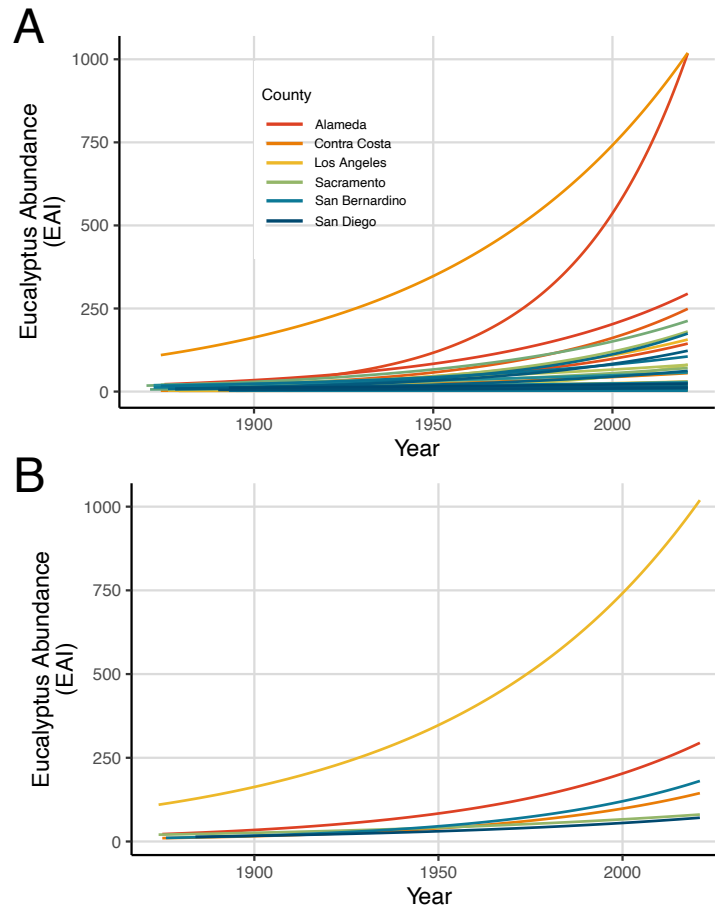


Figure S10. Predicted *Eucalyptus* abundance over time by county. *Eucalyptus* abundance of each county in each year was estimated using the number of articles mentioning relevant search terms in each county in each year (See Table S3). Counts for interim years were predicted using exponentially transformed predictions of a linear model of the log number of articles by year fit to the data. The above figures show predicted count of articles as a function of year for all counties (A) and for six relevant counties (B). *Eucalyptus* density increases exponentially over time in California, with exponential growth happening locally. Notice that Los Angeles County (Southern California) and Alameda County (Northern California) show the earliest increases in *Eucalyptus* search term usage.

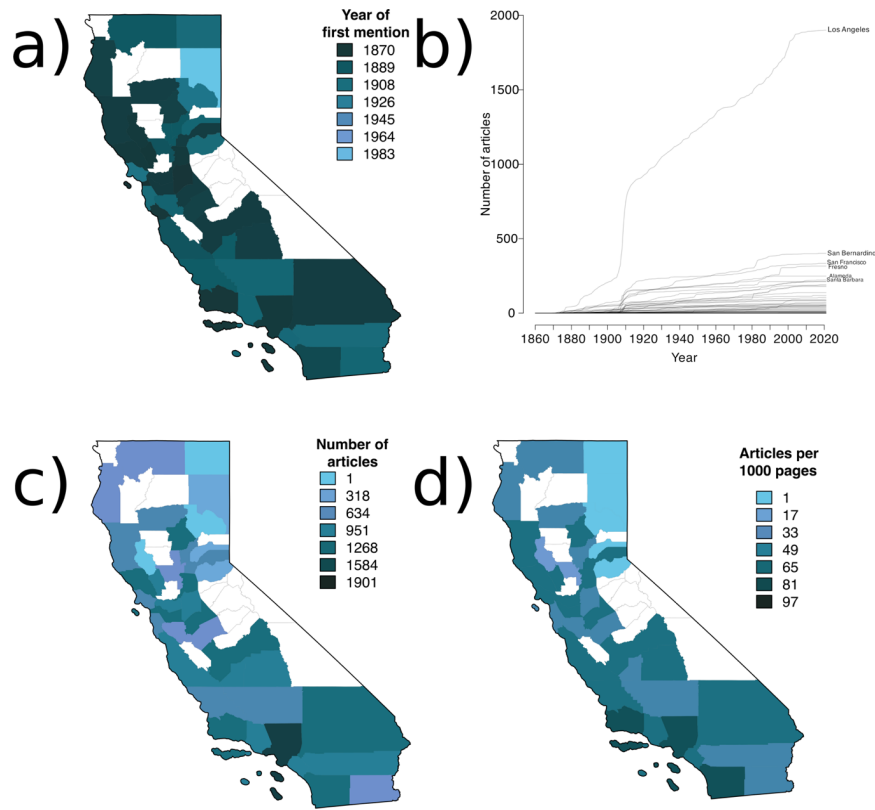


Figure S11. Spatial distribution of search term counts by county. All counts are based on the number of articles mentioning relevant search terms in each county in each year (See Table S3). A. Year of first mention for *Eucalyptus*-related terms. B. Number of articles with mentions over time by county. C. Number of articles with mentions by county. D. Number of articles with mentions per 1000 pages. Search term density increases in southern counties first with local increases in density. Notice in panels B. and C., high density occurs across the state in the early 20th century.

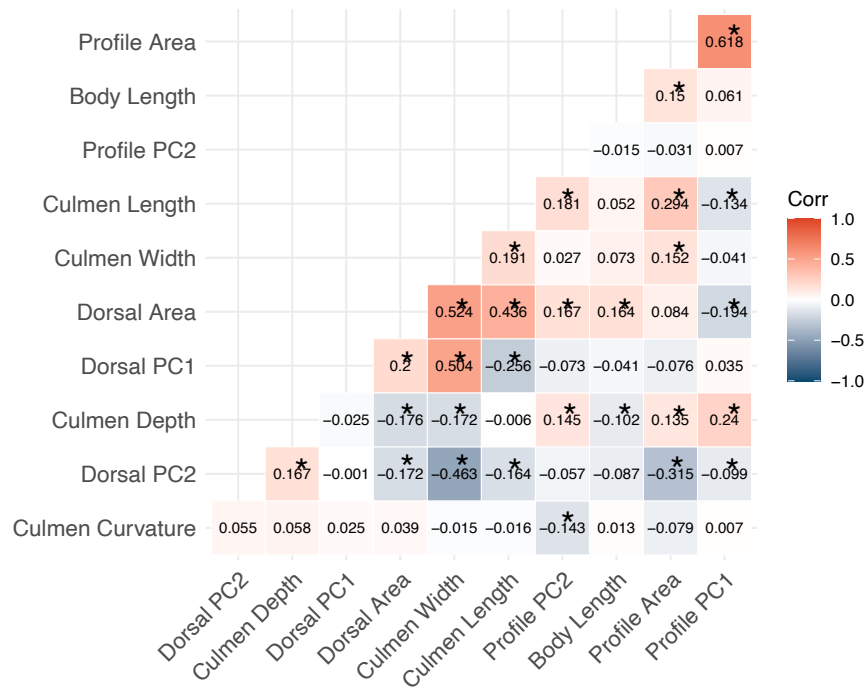


Figure S12. Correlation matrix of measured phenotypic traits. Missing values were imputed by calculating the mean of the non-missing values for each variable, then replacing missing values with that mean. ‘*’ indicate significant correlations at $p < 0.05$ using a linear regression model. Phenotypes that did not meet assumptions of normality were transformed into normalized variables. Body length was not significantly correlated with bill length or width and was not included as a covariate in linear mixed models. Likewise, culmen length was not correlated with bill curvature and was not included in LMM’s with bill curvature as the response variable.

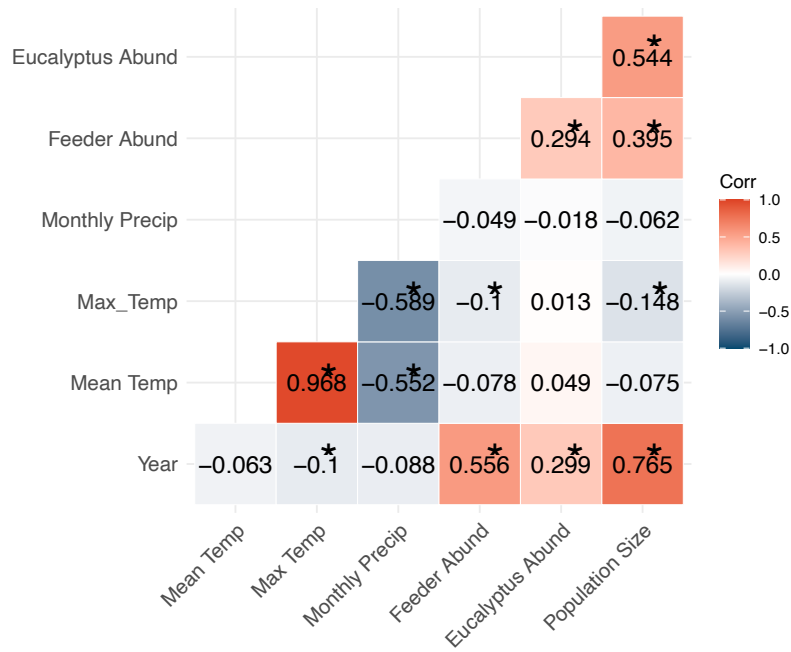


Figure S13. Correlation matrix of year, feeder and *Eucalyptus* abundances, and environmental variables. Missing values were imputed by calculating the mean of the non-missing values for each variable, then replacing missing values with that mean. “*” indicate significant correlations at $p < 0.05$ using a linear regression model. Phenotypes that did not meet assumptions of normality were transformed into normalized variables. Maximum annual temperature was highly correlated with mean annual temperature and was excluded from climate LMM. Human population size, *Eucalyptus* density, and feeder density were all highly correlated, so each used a separate LMM.

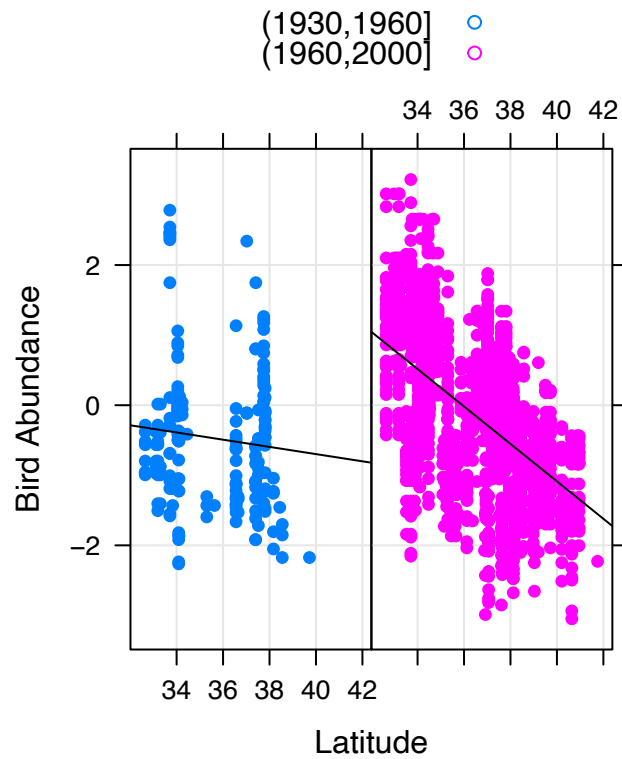


Figure S14. Xyplot of bird density by latitude for the intervals 1930-1960 (blue) and 1961-2000 (pink). Bird density was estimated using all available Christmas Bird Count (CBC) data for California and calculating the number of AH reports per party-hour (following (48) and (49)). Bird population density increases over time in the state of California, and does so more rapidly at southern latitudes in the latter half of the century.

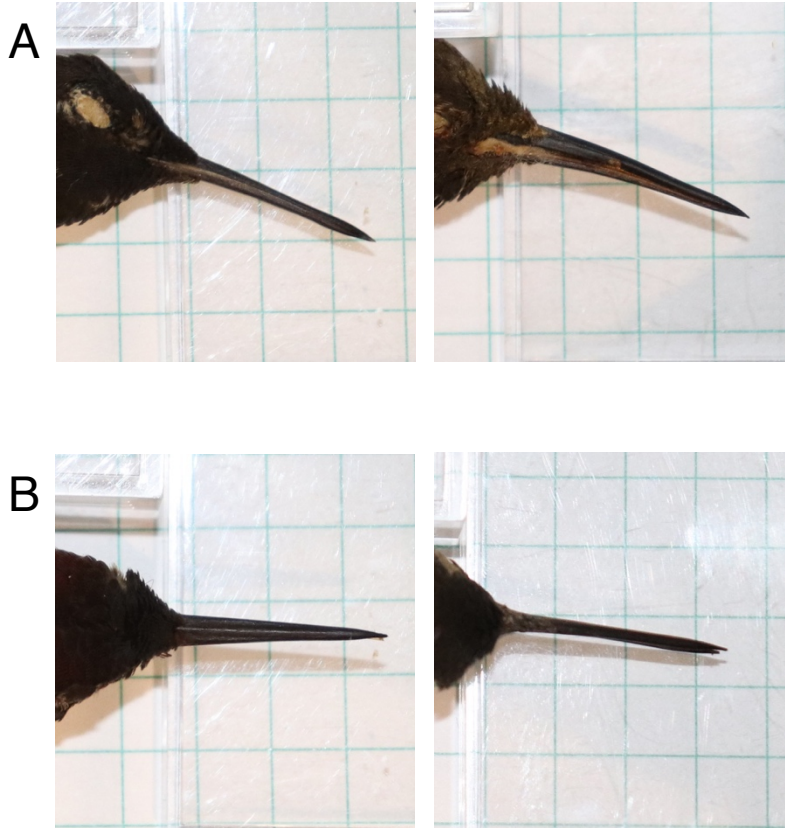


Figure S15. Representative bill images of the main axis (PC1) of profile and dorsal principal component variation. A. Left image: example of a ‘low’ profile PC1 value. This bird has the “feeder-associated” bill with the distinctive bulge at the bill tip, constriction towards the bill center, and a lower depth relative to length along the bill. The right image is an example of a ‘high’ profile PC1 value, indicative of the historic state. B. Left image: example of a ‘high’ dorsal PC1 value indicative of a “feeder-associated” male bill. This shape has the characteristic triangular shape with a higher width to length ratio along the bill length, creating the distinctive point. In the right image is an example of a ‘high’ dorsal PC1 value, representing the historic state of male bill shape.

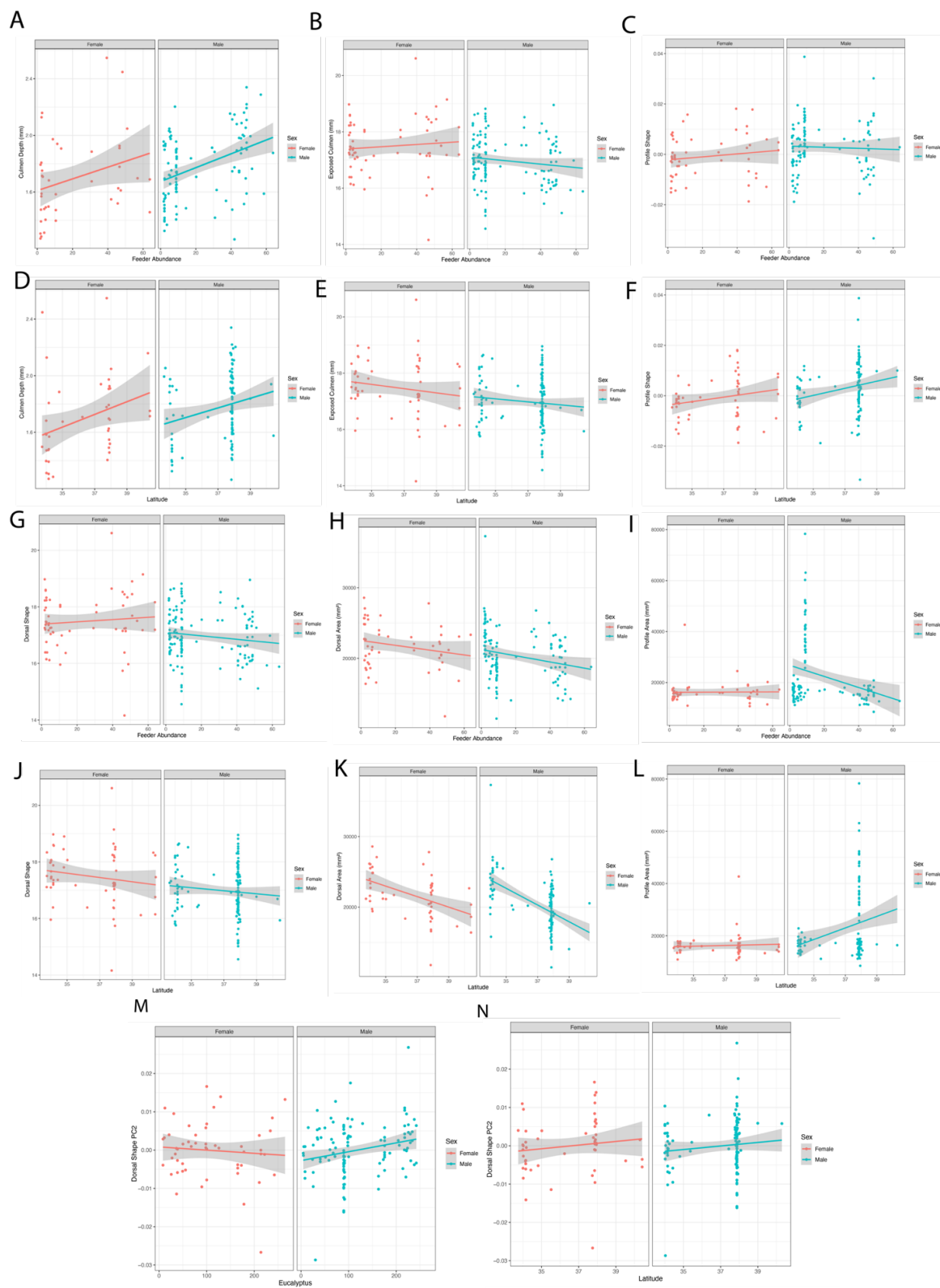


Figure S16. Xyplots depicting the bill phenotypes as a function of latitude or resource density, grouped by sex. A. Culmen depth (mm) as a function of feeder abundance. B. Culmen length (mm) as a function of feeder abundance. C. Profile shape PC1 as a function of feeder abundance. D. Culmen depth (mm) as a function of latitude. E. Culmen length (mm) as a function of latitude. F. Profile shape PC1 as a function of latitude. G. Dorsal shape PC1 as a function of feeder abundance. H. Dorsal area (mm²) as a function of feeder abundance. I. Profile area (mm²) as a function of feeder abundance. J. Dorsal shape PC1 as a function of latitude. K. Dorsal area (mm²) as a function of latitude. L. Profile area (mm²) as a function of latitude. M. Dorsal shape PC2 as a function of *Eucalyptus* density. N. Dorsal shape PC2 as a function of latitude. Xyplots do not control for other covariates and should be interpreted with caution. Some phenotypes display opposing patterns between gradients which may themselves be correlated, such that xyplots display artifactual patterns indicative of associations with multiple silent variables.

Table S1. List of preparators or pairs of preparators and the number of specimens prepared. Due to the large number of preparators, this needed to be included as a random effect in LMMs on body length.

Preparators	Number of Specimens	Preparators	Number of Specimens
Joseph Grinnell	53	G. Covel	1
Francis S. L. Williamson	43	Halstead G. White	1
Christopher J. Clark	27	Harvey M. Hall	1
unknown	24	Hilda W. Grinnell	1
F. Gary Stiles	16	Ioana Seritan	1
Alden H. Miller	13	J. E. Green	1
Allan C. Brooks Jr.	9	J. Van Remsen Jr.	1
Mauricio R. Vazquez	9	Janet C. Fang	1
F. O. Johnson	8	Jean M. Linsdale	1
Chester C. Lamb	7	Jean Mattos-Reano	1
W. B. Judson	7	Jean Mattos-Reano, Jeff T. Wilcox	1
Bernard Bailey	6	Jean Mattos-Reano, Keith Hansen	1
Ralph Arnold [dup]	6	Jeff T. Wilcox, Brad D. McCammack	1
Walter P. Taylor	6	Jessica A. Castillo	1
J. E. Law	5	Jessica Le	1
J. M. Willard	5	John Hornung	1
James F. Lynch	5	Joseph Grinnell, Charles L. Camp	1
Harry S. Swarth	4	Joseph Grinnell, Donald D. McLean	1

John R. Pemberton	4	Joseph Grinnell, Hilda W. Grinnell	1
Ralph E. Ellis Jr.	4	Joseph S. Dixon	1
Richard C. McGregor	4	Joseph T. Marshall Jr., Hope Gladding	1
Robert D. Moore	4	Joshua V. Penalba	1
Teresa J. Feo	4	Kristina Yamamoto	1
Walter E. Bryant	4	Kristina Yamamoto, Jeff T. Wilcox	1
Clark P. Streator	3	Lauryn S. Benedict	1
E. B. Richards	3	Linda S. Hale, J. Van Remsen Jr.	1
Fletcher G. Palmer	3	Malcolm Jollie, A. Robinson	1
J. G. Cooper	3	Martha A. Cerda	1
Joseph T. Marshall Jr.	3	Monica J. Albe, Karen M. C. Rowe	1
Louise Kellogg	3	Monica J. Albe, Sharon W. Lee	1
Monica J. Albe	3	Nassima M. Bouzid	1
Theresa L. Wong	3	Paul D. Hurd Jr.	1
Ward C. Russell	3	R. D. Jennings	1
Charles G. Sibley	2	Ralph Minor	1
Christopher C. Witt	2	Richard C. Banks, Donald R. Medina	1
Donald V. Hemphill	2	Robert A. Norris	1
Ernest D. Clabaugh	2	Robert T. Orr, Henry W. Carriger	1
Frank Stephens	2	Robert W. Storer	1
Frederick H. Test, J. H. Mitchell	2	Rosa A. Jimenez	1

Jeff T. Wilcox	2	Sabrina Horrack	1
Juan L. Parra	2	Sampath Lokugalappatti	1
W. Otto Emerson	2	Seth B. Benson	1
Adrian J. Van Rossem	1	Sina Amini	1
Albert C. Hawbecker	1	Steven W. Cardiff	1
Allan C. Brooks Jr., J. E. Law	1	Teresa J. Feo, Christopher J. Clark	1
Andrea J. Jesse, Keith Hansen	1	Verna C. Bowie	1
C. H. Richardson Jr.	1	Violet Kimzey	1
C. S. Mueller	1	Walter D. Koenig, L. Gould	1
Carla Cicero	1	Walter D. Koenig, Mal Raff	1
Chester C. Lamb, E. Wilbur	1	Walter P. Taylor, N. B. Stern	1
Chester C. Lamb, T. A. Story	1	Ward C. Russell, E. Turner	1
Christopher J. Clark, Jennifer Marks	1	Ward C. Russell, Otto May	1
Cynthia Y. Wang-Claypool	1	Ward C. Russell, William E. Ferguson	1
Dawson A. Feathers	1	William H. Behle	1
Donald D. McLean	1	William H. Behle, Gordon L. Bolander	1
E. Adams	1	Zachary R. Hanna	1
Emanuel Fritz	1	Zachary R. Hanna, Corinne Lambden	1
Emmet T. Hooper	1		
F. Gary Stiles, James F. Parham	1		
F. J. Pember	1		

F. W. Bancroft	1		
Fletcher G. Palmer, Joseph Grinnell	1		
Frederick W. Koch	1		

Table S2. Summary statistics for all measured phenotypes in museum specimens including the minimum, maximum, mean, median, 1st and 3rd quartile, and standard deviation.

Measurement	Min (mm)	1st Qu (mm)	Median (mm)	Mean (mm)	3rd Qu (mm)	Max (mm)	N	SD
Culmen Depth	1.265	1.515	1.673	1.701	1.893	2.548	282	0.2403
Culmen Length	12.6	16.55	17.19	17.17	17.81	20.61	368	1.003
Culmen Width	1.106	1.641	1.805	1.787	1.96	2.462	369	0.2365
Culmen	5.331	8.852	9.511	9.775	10.427	14.815	367	1.397
Body Length	58.09	69.01	71.87	72.11	74.98	83.9	335	4.156
Wing Chord	42.3	48.38	49.12	49.03	49.76	59.44	356	1.371

Measurement	Min (1/r mm)	1st Qu (1/r mm)	Median (1/r mm)	Mean (1/r mm)	3rd Qu (1/r mm)	Max (1/r mm)	N	SD
Culmen	-	0.00754	0.0096	0.00979	0.01242	0.02653	353	0.004148
Curvature	0.01118							

Measurement	Min (g)	1st Qu (g)	Median (g)	Mean (g)	3rd Qu (g)	Max (g)	N	SD
Weight	2.2	3.65	4	4.016	4.3	5.8	147	0.5242

Measurement	Min (SD)	1st Qu (SD)	Median (SD)	Mean (SD)	3rd Qu (SD)	Max (SD)	N	SD
Profile PC1	-0.03333	-0.00669	-0.00081	0	0.00598	0.03923	311	0.009956
Profile PC2	-0.01337	-0.00322	0.00015	0	0.00277	0.01337	311	0.004289
Profile PC3	-0.00862	-0.00175	0.00003	0	0.00159	0.01224	311	0.00283
Dorsal PC1	-0.03999	-0.00777	-0.00039	0	0.00648	0.08102	330	0.01288
Dorsal PC2	-0.02868	-0.00343	-0.00007	0	0.00366	0.02681	330	0.006462
Dorsal PC3	-0.02415	-0.00203	0.00007	0	0.00238	0.01428	330	0.003915

Table S3. Search terms used to identify newspapers articles referring to artificial or non-native nectar sources for hummingbirds in California from 1860-2020. The vertical bar (|) indicates separate but nearly identical search terms where the only distinction is ‘hummingbird’ as a single word or as two words (‘humming bird’) due to change in frequency of use over time.

Search type	Search term(s)
Hummingbird feeders	"hummingbird feeder" "humming bird feeder"
	hummingbird "backyard feeder" "humming bird" "backyard feeder"
	"hummingbird feeding tube" "humming bird feeding tube"
	"hummingbird station" "humming bird station"
	hummingbird "sugar water" "humming bird" "sugar water"
	"hummingbird syrup" "humming bird syrup"
	"hummy-bird"
	"lure hummingbirds" feeder
<i>Eucalyptus</i> plantations	"planted <i>Eucalyptus</i> "
	" <i>Eucalyptus</i> rows"
	"rows <i>Eucalyptus</i> "
	" <i>Eucalyptus</i> plantation"
	"logging <i>Eucalyptus</i> "
	" <i>Eucalyptus</i> logging"
	"blue gum tree"
	"red gum tree"
	" <i>Eucalyptus</i> timber" harvest
	" <i>Eucalyptus</i> planted"

Table S4. Total counts of observed and predicted feeder and *Eucalyptus* abundance, partitioned by county. FAI = Feeder Abundance Index, EAI = *Eucalyptus* Abundance Index. Counts for interim years with no data or a “false zero” were predicted using exponentially transformed predictions of a linear model of the log number of articles by year fit to the data.

County	Subsetting Feeder Abundance (FAI)	Total Feeder Abundance (FAI)	Subsetting <i>Eucalyptus</i> Abundance (FAI)	Total <i>Eucalyptus</i> Abundance (EAI)
Alameda	66	123	182	249
Alpine	NA	0	NA	0
Amador	NA	0	NA	0
Baja California	0	NA	0	NA
Butte	NA	281	NA	212
Calaveras	NA	0	NA	0
Colusa	NA	0	NA	0
Contra Costa	12	134	3	66
Del Norte	NA	0	NA	0
El Dorado	NA	0	NA	2
Fresno	NA	236	NA	316
Glenn	NA	0	NA	0
Humboldt	NA	44	NA	14
Imperial	NA	0	NA	8
Inyo	NA	0	NA	0
Kern	0	36	3	46
Kings	NA	79	NA	86
Lake	0	0	0	1
Lassen	NA	6	NA	2

Los Angeles	10	1404	849	1901
Madera	NA	0	NA	0
Marin	0	51	0	24
Mariposa	0	0	0	0
Mendocino	3	114	0	29
Merced	NA	13	NA	13
Modoc	NA	1	NA	1
Mono	NA	0	NA	0
Monterey	3	88	3	106
Napa	0	106	2	93
Nevada	0	2	2	5
Orange	2	15	4	84
Pima	0	NA	0	NA
Placer	0	159	0	37
Plumas	NA	23	NA	1
Riverside	20	209	0	72
Sacramento	1	893	2	214
San Benito	0	0	0	0
San Bernardino	28	359	91	402
San Diego	0	374	4	179

San Francisco	0	133	4	334
San Joaquin	NA	39	NA	55
San Luis Obispo	0	55	0	53
San Mateo	0	59	0	50
Santa Barbara	10	170	49	223
Santa Clara	2	7	1	11
Santa Cruz	0	241	42	137
Shasta	0	0	0	0
Sierra	NA	1	NA	0
Siskiyou	NA	1	NA	8
Solano	0	3	0	0
Sonoma	0	327	3	192
Stanislaus	0	634	0	184
Sutter	NA	0	NA	19
Tehama	0	1	0	44
Trinity	NA	0	NA	0
Tulare	0	83	0	119
Tuolumne	NA	0	NA	0
Ventura	0	47	1	55
Yolo	NA	0	NA	10

Yuba	0	1	1	35
Yuma	0	NA	0	NA

Table S5. Culmen length MCMCglmm output summaries. A ‘.’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

	Variable	Effect	Posterior Mean	Lower 95% Credible Interval	Upper 95% Credible Interval	Posterior Mode	Effective Sample Size	pMCMC	Significance
Culmen Length Population DIC = 799.1361	Intercept	fixed	1.20E+01	2.38E+00	2.17E+01	9.55E+00	2000	0.016	*
	Year	fixed	-6.16E-03	-1.13E-02	-1.23E-03	-6.17E-03	2000	0.017	*
	Sex	fixed	-2.35E-01	-5.13E-01	2.86E-02	-2.30E-01	2000	0.093	.
	Age	fixed	1.07E-01	-2.10E-01	4.11E-01	1.76E-01	2000	0.499	
	Population	fixed	6.73E-07	2.15E-07	1.13E-06	6.66E-07	2000	0.003	**
	Sex:Population	fixed	-4.38E-07	-8.59E-07	-2.17E-08	-3.97E-07	2000	0.044	*
	County	random	1.21E-01	1.04E-02	2.63E-01	6.00E-02	2000	NA	
	Residuals	residual	7.62E-01	6.36E-01	8.84E-01	7.46E-01	2450	NA	
Culmen Length Feeder Abundance DIC = 452.2622	Intercept	fixed	1.83E+01	2.40E+00	3.37E+01	1.85E+01	2074	0.017	*
	Year	fixed	-9.42E-03	-1.74E-02	-1.09E-03	-9.47E-03	2327	0.018	*
	Sex	fixed	-3.18E-01	-7.03E-01	9.59E-02	-3.35E-01	1876	0.128	
	Age	fixed	2.47E-01	-1.28E-01	6.54E-01	1.83E-01	2000	0.218	
	Feeder Abundance	fixed	2.19E-02	1.66E-05	4.30E-02	1.69E-02	2000	0.041	*
	Sex:Feeder Abundance	fixed	-5.78E-03	-2.42E-02	1.08E-02	-2.44E-03	2082	0.497	

<i>Culmen Length </i> <i>Eucalyptus</i> <i>Abundance</i> <i>DIC = 698.9377</i>	County	ran	8.98E-			1.78E-		
		do	02	1.04E-08	3.14E-01	03	2000	NA
	Residuals	m						
		resi	8.56E-			8.68E-		
		dua	01	6.64E-01	1.05E+00	01	2000	NA
		l						
	Intercept	fixe	6.42E+			7.80E+		0.17
		d	00	-3.19E+00	1.52E+01	00	2000	2
	Year	fixe	-3.29E-			-4.27E-		0.17
		d	03	-7.85E-03	1.67E-03	03	2000	7
	Sex	fixe	-2.25E-			-3.07E-		0.26
		d	01	-5.89E-01	2.09E-01	01	2000	8
	Age	fixe	2.12E-			1.39E-		0.23
		d	01	-1.20E-01	5.61E-01	01	2000	7
	<i>Eucalyptus</i> Abundance	fixe	3.50E-			3.68E-		0.11
		d	03	-1.01E-03	7.92E-03	03	2000	8
	Sex: <i>Eucalyptu</i> s Abundance	fixe	-2.04E-			-1.83E-		0.32
		d	03	-5.76E-03	1.86E-03	03	2134	1
	County	ran						
		do	1.62E-	2.39E-05	3.79E-01	1.31E-	2190	NA
<i>Culmen Length </i> <i>Climate</i> <i>DIC = 709.3047</i>	Intercept	m						
		resi	7.81E-			7.60E-		
	Residuals	dua	01	6.51E-01	9.29E-01	01	2000	NA
		l						
	Intercept	fixe	2.27E+			2.16E+		0.52
		d	00	-4.31E+00	9.19E+00	00	2000	1
	Year	fixe	-1.32E-			-1.68E-		0.47
		d	03	-5.07E-03	1.94E-03	03	2495	9
	Sex	fixe	-4.21E-			-4.10E-		0.00
		d	01	-6.69E-01	-1.95E-01	01	2000	1 ***
	Age	fixe	1.07E-			1.38E-		0.51
		d	01	-2.26E-01	4.30E-01	01	2000	7
	Mean Temperature	fixe	3.16E-	1.58E-03	6.11E-02	3.23E-	2000	0.03 9 *

<i>Culmen Length</i> <i>Base Model</i> <i>DIC = 828.2516</i>	Monthly	fixed	1.57E-03	-1.11E-04	3.42E-03	1.60E-03	2000	0.079
	Precipitation	random	1.28E-01	9.59E-03	2.79E-01	6.72E-02	2377	NA
	County	fixed	7.27E-01	6.07E-01	8.56E-01	7.00E-01	2000	NA
	Residuals	fixed	-6.68E-01	-1.03E+01	9.93E+00	-2.06E-01	2000	0.89
	Intercept	fixed	4.72E-04	-4.99E-03	5.47E-03	2.94E-04	2000	0.859
	Year	fixed	5.62E+00	-6.32E+00	1.62E+01	6.68E+00	1697	0.35
	Sex	fixed	1.34E-01	-1.69E-01	4.50E-01	1.60E-01	1919	0.38
	Age	fixed	-3.08E-03	-8.54E-03	3.08E-03	-3.08E-03	1694	0.323
	Year:Sex	fixed	1.14E-01	1.04E-02	2.52E-01	7.78E-02	2000	NA
	County	fixed	7.79E-01	6.54E-01	9.08E-01	7.62E-01	2000	NA
	Residuals	fixed						
		random						
		fixed						
		random						

Table S6. Culmen width MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

				Lower 95%	Upper 95%		Effective	pM	
	Variable	Effect	Posterior Mean	Credible Interval	Credible Interval	Posterior Mode	Sample Size	CM	Significance
				Interval	Interval	r Mode		C	
Culmen Width		fixed	6.71E+00			4.64E+00		0.21	
Population	Intercept	d	00	-3.01E+00	1.75E+01	00	2000	5	
		fixed	-3.44E-03			-3.54E-03		0.21	
DIC = 834.1051	Year	d	03	-8.72E-03	1.95E-03	03	2000	7	
		fixed	-7.11E-03			-8.38E-03		0.60	
	Sex	d	02	-3.53E-01	2.06E-01	02	2000	9	
		fixed	1.41E-01			1.42E-01		0.38	
	Age	d	01	-1.72E-01	4.75E-01	01	2000	8	
		fixed	7.70E-02			1.16E-01			
	Population	d	08	-3.75E-07	6.13E-07	07	2000	0.74	
	Sex:Population	fixed	-1.55E-07			-2.01E-07		0.47	
	n	d	07	-6.25E-07	2.83E-07	07	2000	9	
	ran								
	do		1.15E-01			5.84E-01			
	County	m	01	1.70E-03	2.77E-01	02	1787	NA	
	resi								
	dua		8.47E-01			8.48E-01			
	Residuals	l	01	7.07E-01	9.90E-01	01	2000	NA	
Culmen Width		fixed	1.15E+00			1.44E+00		0.14	
Feeder Abundance	Intercept	d	01	-3.35E+00	2.75E+01	01	2000	1	
		fixed	-5.92E-03			-5.15E-03		0.14	
DIC = 456.0205	Year	d	03	-1.42E-02	1.78E-03	03	2000	2	
		fixed	1.01E-02			5.30E-02		0.63	
	Sex	d	01	-3.10E-01	5.35E-01	02	2000	4	
		fixed	5.60E-02			-1.68E-01			
	Age	d	02	-3.54E-01	4.46E-01	02	2074	0.79	
	Feeder	fixed	4.94E-02			4.30E-02		0.62	
	Abundance	d	03	-1.40E-02	2.56E-02	03	2150	9	
	Sex:Feeder	fixed	-8.32E-03			-5.81E-03			
	Abundance	d	03	-2.50E-02	9.63E-03	03	2000	0.36	

Culmen Width Eucalyptus Abundance DIC = 720.0719	ran do 6.26E- 1.67E-							
	County	m	02	6.86E-10	2.51E-01	03	2000	NA
	resi dua 8.69E- 8.50E-							
	Residuals	l	01	6.90E-01	1.07E+00	01	1853	NA
	fixe 4.37E+ 6.38E+ 0.43							
	Intercept	d	00	-6.54E+00	1.57E+01	00	1925	3
	fixe -2.25E- -2.13E- 0.43							
	Year	d	03	-8.12E-03	3.34E-03	03	1930	8
	fixe -1.52E- -5.89E- 0.92							
	Sex	d	02	-4.27E-01	3.80E-01	02	2000	8
	fixe 1.08E- 6.57E- 0.54							
	Age	d	01	-2.15E-01	4.86E-01	02	1558	3
	Eucalyptus	fixe	2.18E-			6.16E-		0.88
	Abundance	d	04	-4.68E-03	5.24E-03	04	2000	6
	Sex:Eucalyptu	fixe	-1.40E-			-9.77E-		0.46
	s Abundance	d	03	-5.09E-03	2.70E-03	04	2000	7
	ran do 2.20E- 7.50E-							
	County	m	01	4.78E-13	6.05E-01	02	1940	NA
	resi dua 8.21E- 7.93E-							
	Residuals	l	01	6.81E-01	9.75E-01	01	2000	NA
Culmen Width Climate DIC = 751.6538	fixe 1.01E+ 9.57E+ 0.00							
	Intercept	d	01	3.06E+00	1.72E+01	00	2000	5 **
	fixe -5.42E- -4.18E- 0.00							
	Year	d	03	-9.03E-03	-1.79E-03	03	2000	2 **
	fixe -1.24E- -1.39E- 0.35							
	Sex	d	01	-3.64E-01	1.48E-01	01	2000	1
	fixe 9.31E- 5.80E- 0.61							
	Age	d	02	-2.38E-01	4.44E-01	02	2000	3
	Mean	fixe	3.04E-			2.91E-		0.06
	Temperature	d	02	-1.61E-03	6.14E-02	02	2000	6 .

<i>Culmen Width Base Model</i> <i>DIC = 855.8904</i>	Monthly	fixe	7.73E-			7.64E-		0.40	
	Precipitation	d	04	-1.04E-03	2.78E-03	04	2000	7	
		ran							
		do	6.95E-			1.95E-			
	County	m	02	3.17E-08	2.26E-01	03	2204	NA	
		resi							
		dua	8.60E-			8.22E-			
	Residuals	l	01	7.09E-01	1.01E+00	01	2000	NA	
		fixe	1.20E+			1.21E+		0.02	
	Intercept	d	01	1.45E+00	2.19E+01	01	1917	3 *	
		fixe	-6.17E-			-6.62E-		0.02	
	Year	d	03	-1.12E-02	-6.54E-04	03	1918	3 *	
		-				-			
		fixe	6.29E+			4.47E+		0.31	
	Sex	d	00	-1.92E+01	5.34E+00	00	2000	4	
		fixe	1.75E-			1.28E-		0.29	
	Age	d	01	-1.49E-01	5.05E-01	01	2376	4	
		fixe	3.17E-			3.02E-		0.32	
	Year:Sex	d	03	-2.86E-03	9.81E-03	03	2000	5	
		ran							
		do	9.78E-			4.95E-			
	County	m	02	4.54E-04	2.49E-01	02	1640	NA	
		resi							
		dua	8.48E-			8.64E-			
	Residuals	l	01	7.16E-01	9.87E-01	01	2000	NA	

Table S7. Culmen depth MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

				Lower 95%	Upper 95%		Effective	pM	
		Effect	Posterior	Credible	Credible	Posterior	Sample	CM	Signifi
		Variable	Mean	Interval	Interval	Mode	Size	C	cance
Culmen Depth Population DIC = 587.29			-			-			
		Intercept	fixe 1.33E+01			1.05E+01		0.01	
			d 01	-2.34E+01	-3.59E+00	01	2000	1	*
		Year	fixe 6.74E-03			6.48E-03		0.01	
			d 03	1.67E-03	1.20E-02	03	2000	4	*
		Sex	fixe 1.46E-01			1.04E-01		0.30	
			d 01	-1.34E-01	4.19E-01	01	2000	8	
		Age	fixe -2.04E-01			-2.85E-01		0.22	
			d 01	-5.13E-01	1.62E-01	01	2000	9	
		Population	fixe 1.03E-06			1.01E-06		0.00	
			d 06	4.49E-07	1.58E-06	06	1967	5	***
		Sex:Population	fixe -9.34E-07			-9.03E-07		0.00	
			d 07	-1.44E-06	-3.66E-07	07	2000	5	***
		County	fixe 4.10E-02			1.12E-02		0.00	
			m 02	1.78E-08	1.22E-01	03	2000	NA	
Culmen Depth Feeder Abundance DIC = 342.7984			-			-			
		Intercept	fixe 2.22E+01			2.24E+01		0.01	
			d 01	-3.88E+01	-6.77E+00	01	2000	4	*
		Year	fixe 1.13E-02			1.15E-02		0.01	
			d 02	3.58E-03	2.02E-02	02	2000	4	*
		Sex	fixe 3.47E-01			3.02E-01		0.13	
			d 01	-1.02E-01	8.14E-01	01	2000	1	
		Age	fixe -3.01E-01			-2.24E-01		0.15	
			d 01	-7.25E-01	1.07E-01	01	2000	0.15	
		Residuals	fixe 6.78E-01			6.71E-01		0.00	
			l 01	5.49E-01	8.02E-01	01	1884	NA	

	Feeder	fixe	2.06E-			1.87E-		0.07
	Abundance	d	02	-3.56E-03	4.16E-02	02	1745	4
	Sex:Feeder	fixe	-2.51E-			-2.18E-		0.01
	Abundance	d	02	-4.32E-02	-4.76E-03	02	2001	3
		ran						
		do	5.40E-			2.37E-		
	County	m	02	2.51E-07	1.94E-01	03	2000	NA
		resi						
		dua	7.81E-			7.69E-		
	Residuals	l	01	5.81E-01	9.61E-01	01	2000	NA
Culmen Depth			-			-		<
Eucalyptus		fixe	2.15E+			2.20E+		0.00
Abundance	Intercept	d	01	-2.87E+01	-1.38E+01	01	2000	5

								<
		fixe	1.12E-			1.11E-		0.00
DIC = 517.5841	Year	d	02	7.22E-03	1.50E-02	02	2000	5

		fixe	-1.97E-			-3.07E-		0.32
	Sex	d	01	-6.18E-01	1.89E-01	01	1817	5
		fixe	-2.50E-			-1.83E-		
	Age	d	01	-6.18E-01	1.40E-01	01	1870	0.21
	Eucalyptus	fixe	-1.92E-			-2.31E-		0.30
	Abundance	d	03	-5.45E-03	1.67E-03	03	2000	3
	Sex:Eucalyptu	fixe	1.33E-			9.88E-		0.50
	s Abundance	d	03	-2.54E-03	5.09E-03	04	2000	1
		ran						
		do	3.00E-			9.48E-		
	County	m	02	2.20E-08	9.89E-02	04	2000	NA
		resi						
		dua	7.14E-			7.22E-		
	Residuals	l	01	5.77E-01	8.49E-01	01	2000	NA
Culmen Depth			-			-		<
Climate		fixe	2.16E+			2.28E+		0.00
	Intercept	d	01	-2.78E+01	-1.50E+01	01	1799	5

<i>DIC = 542.026</i>								<	
		fixe	1.15E-			1.17E-		0.00	
	Year	d	02	8.24E-03	1.47E-02	02	2000	5	***
		fixe	-1.26E-			-1.33E-		0.33	
	Sex	d	01	-4.08E-01	1.12E-01	01	2000	8	
		fixe	-1.51E-			-1.93E-		0.40	
	Age	d	01	-5.21E-01	1.78E-01	01	2000	5	
	Mean	fixe	-4.06E-			-3.30E-		0.00	
	Temperature	d	02	-6.95E-02	-7.92E-03	02	2000	8	*
	Monthly	fixe	-4.65E-			-5.26E-		0.62	
	Precipitation	d	04	-2.38E-03	1.37E-03	04	2000	5	
		ran							
		do	1.44E-			3.96E-			
	County	m	02	1.13E-07	5.60E-02	04	2000	NA	
		resi							
		dua	7.03E-			6.93E-			
	Residuals	l	01	5.69E-01	8.49E-01	01	2000	NA	
<i>Culmen Depth Base Model</i>								<	
		fixe	2.70E+			2.83E+		0.00	
	Intercept	d	01	-3.79E+01	-1.46E+01	01	2152	5	***
								<	
		fixe	1.39E-			1.46E-		0.00	
	Year	d	02	7.58E-03	1.97E-02	02	2150	5	***
		fixe	9.44E+			1.08E+		0.16	
	Sex	d	00	-4.55E+00	2.20E+01	01	2000	1	
		fixe	-2.96E-			-2.57E-		0.08	
	Age	d	01	-6.37E-01	2.28E-02	01	2000	2	.
		fixe	-4.91E-			-5.61E-		0.15	
	Year:Sex	d	03	-1.11E-02	2.71E-03	03	2000	9	
		ran							
		do	3.30E-			7.01E-			
	County	m	02	2.55E-08	1.05E-01	04	2000	NA	
		resi							
		dua	7.36E-			7.57E-			
	Residuals	l	01	6.11E-01	8.76E-01	01	2000	NA	

Table S8. Culmen curvature MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

			Posteri	Lower 95%	Upper 95%	Posteri	Effective	pM	
		Effe	or	Credible	Credible	or	Sample	CM	Signifi
Variable	ct	Mean	Interval	Interval	Mode	Size	C	cance	
Culmen Curvature Population DIC = 842.0335		fixe	1.15E+			1.04E+		0.03	
	Intercept	d	01	1.12E+00	2.26E+01	01	2000	9	*
		fixe	-5.76E-			-5.30E-		0.04	
	Year	d	03	-1.15E-02	-2.96E-04	03	2000	3	*
								<	
		fixe	-6.46E-			-6.69E-		0.00	
	Sex	d	01	-9.61E-01	-3.57E-01	01	2000	05	***
		fixe	-2.08E-			-2.36E-		0.27	
	Age	d	01	-5.61E-01	1.56E-01	01	2154	5	
		fixe	-8.28E-			4.47E-		0.76	
	Population	d	08	-5.71E-07	4.61E-07	08	2000	5	
	Sex:Population	fixe	4.90E-			5.59E-		0.04	
	n	d	07	2.64E-08	9.64E-07	07	2000	3	*
	ran								
	do		1.22E-			5.53E-			
Culmen Curvature Feeder Abundance DIC = 433.5735	County	m	01	3.11E-07	3.21E-01	02	2000	NA	
	resi								
	dua		9.47E-			9.20E-			
	Residuals	l	01	7.90E-01	1.11E+00	01	2000	NA	
		fixe	6.00E+			2.74E+		0.46	
	Intercept	d	00	-1.02E+01	2.15E+01	00	2000	8	
		fixe	-2.94E-			-1.20E-			
	Year	d	03	-1.09E-02	5.52E-03	03	2000	0.49	
		fixe	-6.53E-			-6.40E-		0.00	
	Sex	d	01	-1.11E+00	-2.27E-01	01	2000	3	**
		fixe	-1.78E-			-2.30E-		0.40	
	Age	d	01	-5.57E-01	2.67E-01	01	1750	6	
	Feeder	fixe	-2.94E-			-1.69E-		0.72	
	Abundance	d	03	-2.38E-02	1.82E-02	03	1762	7	

Culmen Curvature Eucalyptus Abundance DIC = 710.9267 Culmen Curvature Climate DIC = 753.2053	Sex:Feeder	fixe	1.68E-			1.58E-	0.06	
	Abundance	d	02	-1.07E-03	3.41E-02	02	2000	8 .
		ran						
		do	7.61E-			3.34E-		
	County	m	02	4.49E-10	2.68E-01	03	2000	NA
		resi						
		dua	8.36E-			8.26E-		
	Residuals	l	01	6.62E-01	1.03E+00	01	2325	NA
		fixe	5.13E+			5.23E+	0.28	
	Intercept	d	00	-3.97E+00	1.60E+01	00	2608	8
		fixe	-2.58E-			-3.34E-	0.30	
	Year	d	03	-8.14E-03	2.25E-03	03	2600	2
		fixe	-5.60E-			-6.49E-	0.01	
	Sex	d	01	-1.01E+00	-1.26E-01	01	2000	6 *
		fixe	-2.74E-			-3.02E-		
	Age	d	01	-6.24E-01	9.29E-02	01	2000	0.15
	Eucalyptus	fixe	3.74E-			2.01E-	0.87	
	Abundance	d	04	-4.08E-03	5.02E-03	04	2000	5
	Sex:Eucalypt	fixe	2.28E-			1.68E-	0.30	
	us Abundance	d	03	-1.91E-03	6.81E-03	03	2000	6
		ran						
		do	1.52E-			7.87E-		
	County	m	01	6.75E-07	3.71E-01	02	2000	NA
		resi						
		dua	8.82E-			8.60E-		
	Residuals	l	01	7.31E-01	1.04E+00	01	2000	NA
		fixe	4.08E+			5.56E+	0.28	
	Intercept	d	00	-3.15E+00	1.13E+01	00	2132	6
		fixe	-2.22E-			-3.09E-	0.25	
	Year	d	03	-5.94E-03	1.54E-03	03	1849	9
		fixe	-4.46E-			-3.67E-	0.00	
	Sex	d	01	-7.43E-01	-1.75E-01	01	2000	1 ***
		fixe	-2.16E-			-2.32E-	0.25	
	Age	d	01	-5.74E-01	1.57E-01	01	2000	8

<i>Culmen Curvature</i> <i>Base Model</i> <i>DIC = 866.5398</i>	Mean	fixe	2.60E-			3.02E-	0.12
	Temperature	d	02	-4.95E-03	6.30E-02	02	1861 1
	Monthly	fixe	1.73E-			1.85E-	0.07
	Precipitation	d	03	-2.58E-04	3.70E-03	03	2345 9
		ran					
		do	6.83E-			9.14E-	
	County	m	02	7.15E-09	2.21E-01	04	2000 NA
		resi					
		dua	9.50E-			9.53E-	
	Residuals	l	01	7.86E-01	1.12E+00	01	2025 NA
		fixe	6.73E+			5.49E+	0.23
	Intercept	d	00	-4.54E+00	1.73E+01	00	1660 6
		fixe	-3.34E-			-2.69E-	0.26
	Year	d	03	-8.77E-03	2.54E-03	03	1660 4
		-				-	
		fixe	2.17E+			1.21E+	0.76
	Sex	d	00	-1.47E+01	1.20E+01	00	2000 2
		fixe	-1.73E-			-2.07E-	0.34
	Age	d	01	-5.35E-01	1.79E-01	01	1827 7
		fixe	8.80E-			3.99E-	0.80
	Year:Sex	d	04	-6.52E-03	7.30E-03	04	2000 4
		ran					
		do	6.29E-			1.75E-	
	County	m	02	1.19E-06	2.04E-01	03	2000 NA
		resi					
		dua	9.74E-			9.36E-	
	Residuals	l	01	8.21E-01	1.15E+00	01	2000 NA

Table S9. Culmen length/width ratio MCMCglmm output summaries. A ‘.’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

			Posterior Effect Variable	Mean	Lower 95% Credible Interval	Upper 95% Credible Interval	Posterior or Mode	Effective Sample Size	pM CM C	Signif icanc e
Culmen Length/Width Population DIC = 825.1292			-				-			
		fixed		2.79E+			3.39E+		0.56	
	Intercept	d		00	-1.21E+01	6.81E+00	00	2169	7	
		fixed		1.44E-			1.73E-		0.57	
	Year	d		03	-3.49E-03	6.33E-03	03	2164	3	
		fixed		-8.32E-			-8.13E-		0.55	
	Sex	d		02	-3.65E-01	1.78E-01	02	2000	8	
		fixed		-9.38E-			-4.45E-		0.95	
	Age	d		03	-3.48E-01	2.86E-01	02	2000	8	
		fixed		1.15E-			1.35E-		0.63	
	Population	d		07	-3.57E-07	5.90E-07	07	2000	8	
	Sex:Population	fixed		2.17E-			8.56E-		0.93	
		d		08	-4.09E-07	4.74E-07	08	2000	9	
		random								
Culmen Length/Width Feeder Abundance DIC = 457.0146				4.09E-			1.04E-			
	County	m		02	8.94E-09	1.39E-01	03	1556	NA	
		residual								
		fixed		8.50E-			8.71E-			
	Residuals	l		01	7.16E-01	9.93E-01	01	2000	NA	
		fixed		2.20E+			3.43E-		0.77	
	Intercept	d		00	-1.58E+01	1.46E+01	01	2173	9	
		fixed		1.13E-			1.07E-		0.77	
	Year	d		03	-7.66E-03	8.19E-03	04	2175	9	
		fixed		-2.45E-			-2.71E-		0.25	
	Sex	d		01	-6.69E-01	1.82E-01	01	2000	5	
		fixed		1.08E-			1.18E-		0.59	
	Age	d		01	-2.59E-01	5.24E-01	01	2138	8	
	Feeder	fixed		3.21E-			3.73E-		0.73	
	Abundance	d		03	-1.72E-02	2.22E-02	03	2433	7	

Culmen Length/Width Eucalyptus Abundance DIC = 708.9471	Sex:Feeder	fixe	6.91E-			8.42E-	0.42	
	Abundance	d	03	-1.02E-02	2.34E-02	03	2000	9
		ran						
		do	5.16E-			1.09E-		
	County	m	02	9.72E-09	2.18E-01	03	1856	NA
		resi						
		dua	8.86E-			8.39E-		
	Residuals	l	01	6.92E-01	1.09E+00	01	2000	NA
				-		-		
		fixe	3.24E+			4.97E+		0.47
	Intercept	d	00	-1.19E+01	7.55E+00	00	1842	8
		fixe	1.69E-			2.31E-		0.47
	Year	d	03	-3.91E-03	6.22E-03	03	1847	4
		fixe	-1.04E-			-1.26E-		
	Sex	d	01	-4.99E-01	2.79E-01	01	1649	0.62
		fixe	6.51E-			5.47E-		0.70
	Age	d	02	-2.86E-01	4.16E-01	02	2000	5
	Eucalyptus	fixe	-2.44E-			-6.58E-		0.92
Abundance	d	05	-4.57E-03	5.22E-03	05	2000	2	
Sex:Eucalyptus	fixe	6.63E-			5.56E-		0.75	
Abundance	d	04	-3.14E-03	4.69E-03	04	1709	8	
	ran							
	do	9.63E-			9.49E-			
County	m	02	2.55E-08	3.49E-01	04	2000	NA	
	resi							
	dua	8.23E-			8.02E-			
Residuals	l	01	6.88E-01	9.76E-01	01	2163	NA	
			-		-			
Culmen Length/Width Climate DIC = 742.898		fixe	7.86E+			8.67E+	0.02	
Intercept	d	00	-1.43E+01	-1.53E+00	00	2148	1 *	
	fixe	4.23E-			4.62E-		0.01	
Year	d	03	8.95E-04	7.47E-03	03	2149	4 *	
	fixe	-1.02E-			-1.60E-		0.41	
Sex	d	01	-3.35E-01	1.63E-01	01	2000	7	

<i>Culmen Length/Width</i> <i>Base Model</i> <i>DIC = 849.6512</i>		fixe	2.68E-			1.20E-		0.88	
	Age	d	02	-2.97E-01	3.76E-01	02	2000	5	
	Mean	fixe	-2.03E-			-1.67E-		0.17	
	Temperature	d	02	-5.15E-02	8.31E-03	02	2000	4	
	Monthly	fixe	-4.62E-			-3.51E-		0.64	
	Precipitation	d	04	-2.21E-03	1.51E-03	04	2000	7	
		ran							
		do	2.24E-			8.50E-			
	County	m	02	4.49E-09	8.90E-02	04	1496	NA	
		resi							
		dua	8.52E-			8.27E-			
	Residuals	l	01	7.22E-01	1.01E+00	01	2004	NA	
			-			-			
		fixe	9.74E+			1.01E+		0.07	
	Intercept	d	00	-2.02E+01	7.95E-01	01	1883	3	.
		fixe	5.04E-			5.65E-		0.07	
	Year	d	03	-1.82E-04	1.06E-02	03	1880	4	.
		fixe	5.67E+			8.44E+		0.36	
	Sex	d	00	-6.30E+00	1.69E+01	00	2000	1	
		fixe	-2.97E-			1.67E-		0.85	
	Age	d	02	-3.47E-01	2.72E-01	03	1954	3	
		fixe	-2.94E-			-4.35E-		0.35	
	Year:Sex	d	03	-8.86E-03	3.17E-03	03	2000	9	
		ran							
		do	3.63E-			6.97E-			
	County	m	02	1.42E-10	1.29E-01	04	1839	NA	
		resi							
		dua	8.54E-			8.28E-			
	Residuals	l	01	7.15E-01	9.91E-01	01	2000	NA	

Table S10. Bill profile area MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

				Lower 95%	Upper 95%		Effective	pM	
		Effect	Posterior	Credible	Credible	Posterior	Sample	CM	Signifi
Variable		ct	r Mean	Interval	Interval	r Mode	Size	C	cance
Profile Area Population DIC = 675.9788			-			-		<	
		fixe	2.72E+0			2.60E+0		0.00	
	Intercept	d	1	-3.82E+01	-1.46E+01	1	2000	05	***
								<	
		fixe	1.40E-			1.40E-		0.00	
	Year	d	02	7.42E-03	1.97E-02	02	2000	05	***
		fixe	3.83E-			4.40E-			
	Sex	d	01	8.87E-02	6.66E-01	01	2000	0.01	*
		fixe	3.35E-			1.68E-		0.84	
	Age	d	02	-3.02E-01	3.46E-01	02	1798	5	
								<	
		fixe	-8.85E-			-9.47E-		0.00	
	Population	d	07	-1.43E-06	-3.50E-07	07	2145	05	***
	Sex:Population	fixe	-2.14E-			-2.77E-		0.38	
Profile Area Feeder Abundance DIC = 440.9211	n	d	07	-6.71E-07	2.71E-07	07	2000	3	
		ran							
		do	2.38E-			1.93E-			
	County	m	01	7.96E-02	4.14E-01	01	1873	NA	
		resi	7.43E-			7.34E-			
	Residuals	dual	01	6.15E-01	8.83E-01	01	2000	NA	
			-			-			
		fixe	4.89E+0			4.10E+0		0.00	
	Intercept	d	1	-8.05E+01	-1.81E+01	1	1717	2	**
		fixe	2.52E-			2.64E-		0.00	
	Year	d	02	1.00E-02	4.22E-02	02	1719	2	**
		fixe	7.22E-			8.05E-		0.00	
	Sex	d	01	2.11E-01	1.23E+00	01	2000	3	**
		fixe	1.72E-			9.82E-		0.47	
	Age	d	01	-3.19E-01	6.66E-01	02	2001	3	

<i>Profile Area </i> <i>Eucalyptus</i> <i>Abundance</i> <i>DIC = 634.462</i>	Feeder		fixe	-5.35E-			-4.70E-		0.00	
	Abundance	d	02	-9.26E-02	-1.54E-02	02	1622	3	**	
	Sex:Feeder		fixe	-2.52E-			-2.88E-			
	Abundance	d	02	-4.67E-02	-3.66E-03	02	1695	0.03	*	
			ran							
			do	7.59E-			5.50E-			
	County	m	01	9.34E-02	1.90E+00	01	1731	NA		
			resi	1.04E+0			1.01E+0			
	Residuals	dual	0	7.90E-01	1.30E+00	0	1899	NA		
	<i>Profile Area </i>			-			-			
	<i>Eucalyptus</i>		fixe	1.54E+0			1.46E+0		0.02	
	<i>Abundance</i>		Intercept	d	1	-3.07E+01	-1.13E+00	1	2000	5 *
			fixe	7.82E-			7.81E-			
			Year	d	03	4.57E-04	1.59E-02	03	2000	0.03 *
			fixe	5.75E-			5.08E-		0.02	
			Sex	d	01	1.06E-01	1.09E+00	01	2196	6 *
			fixe	4.85E-			4.90E-		0.80	
			Age	d	02	-3.60E-01	4.44E-01	02	2316	6
	<i>Eucalyptus</i>		fixe	-4.53E-			-3.60E-			
	Abundance	d	03	-1.08E-02	1.90E-03	03	2000	0.16		
	Sex: <i>Eucalyptu</i>		fixe	-2.46E-			-2.39E-		0.28	
	s Abundance	d	03	-6.70E-03	1.87E-03	03	2000	2		
			ran							
			do	3.48E-			2.30E-			
	County	m	01	7.84E-02	7.47E-01	01	2000	NA		
			resi	9.33E-			9.34E-			
	Residuals	dual	01	7.43E-01	1.11E+00	01	2000	NA		
<i>Profile Area </i> <i>Climate</i> <i>DIC = 673.0058</i>			fixe	-2.38E-			3.22E-			
	<i>Climate</i>		Intercept	d	01	-8.48E+00	9.19E+00	01	2000	0.97
			fixe	1.37E-			1.49E-		0.96	
			Year	d	04	-4.58E-03	4.51E-03	03	2000	3
			fixe	2.00E-			2.36E-		0.16	
			Sex	d	01	-9.22E-02	4.72E-01	01	2152	8
			fixe	1.52E-			1.41E-		0.42	
			Age	d	01	-2.11E-01	5.32E-01	01	2000	2

<i>Profile Area Base</i> <i>Model</i> <i>DIC = 726.5316</i>	Mean	fixe	-2.45E-			-2.72E-		0.17
	Temperature	d	02	-5.93E-02	1.07E-02	02	2000	5
	Monthly	fixe	1.52E-			4.41E-		0.87
	Precipitation	d	04	-1.95E-03	2.15E-03	04	2540	3
		ran						
		do	1.59E-			1.20E-		
	County	m	01	4.52E-02	3.10E-01	01	1649	NA
		resi	9.13E-			8.85E-		
	Residuals	dual	01	7.54E-01	1.09E+00	01	2001	NA
			-			-		
		fixe	2.22E+0			5.58E+0		0.71
	Intercept	d	0	-1.38E+01	9.63E+00	0	2000	1
		fixe	9.56E-			2.61E-		0.75
	Year	d	04	-5.13E-03	7.00E-03	03	2000	6
		fixe	-6.19E-			-5.35E-		0.89
	Sex	d	01	-1.50E+01	1.26E+01	01	2000	9
		fixe	6.23E-			2.38E-		0.72
	Age	d	02	-2.67E-01	4.23E-01	02	2000	8
		fixe	4.54E-			1.49E-		0.87
	Year:Sex	d	04	-6.54E-03	7.74E-03	03	2000	5
		ran						
		do	1.33E-			1.25E-		
	County	m	01	4.24E-02	2.51E-01	01	2000	NA
		resi	8.47E-			8.41E-		
	Residuals	dual	01	6.93E-01	9.94E-01	01	2000	NA

Table S11. Profile PC1 MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1. Principal components of bill shape should be unaffected by body size as the geomorphometric methods that generated these PC values remove this effect, so body size was not included as a covariate.

				Lower 95%	Upper 95%				
		Effe	Posterior	Credible	Credible	Posterior	Effective	pM	
		Variable	ct	Interval	Interval	r Mode	Sample	CM	Signifi
			r Mean				Size	C	cance
Profile PC1 Population DIC = 702.5492	Intercept	fixe	1.70E+0			1.52E+0		0.00	
		d	1	-2.67E+01	-4.88E+00	1	2000	4	**
		fixe	8.64E-			7.82E-		0.00	
		d	03	2.41E-03	1.38E-02	03	2000	4	**
		fixe	3.75E-			3.60E-		0.01	
		d	01	7.74E-02	6.52E-01	01	2000	4	*
		fixe	-1.79E-			-2.59E-		0.32	
		d	01	-5.25E-01	1.52E-01	01	2000	7	
		fixe	-4.17E-			-4.27E-			
		d	07	-9.39E-07	1.34E-07	07	2000	0.13	
		fixe	-1.50E-			-1.34E-		0.55	
		d	07	-7.03E-07	2.94E-07	07	2000	8	
		ran							
		do	1.34E-			6.95E-			
Profile PC1 Feeder Abundance DIC = 416.9996	Intercept	m	01	2.00E-02	3.06E-01	02	2765	NA	
		resi	7.82E-			7.37E-			
		dual	01	6.58E-01	9.30E-01	01	2000	NA	
		fixe	3.76E+0			4.07E+0		0.00	
		d	1	-5.49E+01	-2.01E+01	1	2000	05	***
		fixe	1.94E-			2.04E-		0.00	
		d	02	1.06E-02	2.86E-02	02	2000	05	***
		fixe	4.63E-			4.07E-		0.04	
		d	01	4.13E-02	9.47E-01	01	2000	9	*
		fixe	-2.60E-			-2.65E-		0.25	
		d	01	-6.73E-01	1.74E-01	01	1818	2	
		ran							
		do							
		m							
		resi							

<i>DIC = 629.1934</i> <i>Profile PCI </i> <i>Eucalyptus</i> <i>Abundance</i> <i>DIC = 629.1934</i>	Feeder	fixe	-3.17E-			-3.20E-		0.00	
	Abundance	d	02	-5.61E-02	-8.95E-03	02	2000	7	**
	Sex:Feeder	fixe	-1.30E-			-1.43E-		0.20	
	Abundance	d	02	-3.30E-02	6.80E-03	02	2000	2	
		ran							
		do	1.16E-			1.95E-			
	County	m	01	3.77E-07	4.24E-01	03	2000	NA	
		resi	8.53E-			8.02E-			
	Residuals	dual	01	6.61E-01	1.05E+00	01	2222	NA	
				-		-			
		fixe	9.38E+0			8.32E+0		0.10	
	Intercept	d	0	-2.19E+01	2.70E+00	0	1989	9	
		fixe	4.70E-			4.56E-		0.12	
	Year	d	03	-1.52E-03	1.13E-02	03	2011	1	
		fixe	4.27E-			3.33E-		0.06	
	Sex	d	01	-2.24E-02	8.95E-01	01	2000	3	.
		fixe	-1.35E-			-1.48E-		0.50	
	Age	d	01	-5.20E-01	2.27E-01	01	2000	2	
	<i>Eucalyptus</i>	fixe	-1.80E-			-1.53E-		0.53	
	Abundance	d	03	-7.01E-03	3.22E-03	03	2000	3	
	Sex: <i>Eucalyptu</i>	fixe	-8.53E-			-6.31E-		0.70	
<i>s Abundance</i> <i>Profile PCI </i> <i>Climate</i> <i>DIC = 661.7567</i>		d	04	-5.10E-03	3.27E-03	04	2000	5	
		ran							
		do	1.91E-			7.13E-			
	County	m	01	8.79E-03	4.97E-01	02	2000	NA	
		resi	8.58E-			8.51E-			
	Residuals	dual	01	7.02E-01	1.02E+00	01	2692	NA	
				-		-			
		fixe	5.50E+0			4.92E+0		0.18	
	Intercept	d	0	-1.42E+01	1.92E+00	0	2000	6	
		fixe	2.63E-			3.00E-		0.21	
	Year	d	03	-1.27E-03	7.02E-03	03	2000	6	
		fixe	3.24E-			3.10E-		0.02	
	Sex	d	01	6.98E-02	6.11E-01	01	2000	5	*

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Table S12. Profile PC2 MCMCglmm output summaries. A ‘.’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1. Principal components of bill shape should be unaffected by body size as the geomorphometric methods that generated these PC values remove this effect, so body size was not included as a covariate.

				Lower 95%	Upper 95%		Effective		
	Variable	Effect	Posterior Mean	Credible Interval	Credible Interval	Posterior Mode	Sample Size	pMC MC	Significance
Profile PC2		fixed	1.26E+0			1.24E+0		0.02	
Population DIC = 741.069	Intercept	d	1	1.55E+00	2.35E+01	1	2000	7	*
		fixed	-6.75E-			-6.10E-			
	Year	d	03	-1.24E-02	-1.09E-03	03	2000	0.02	*
		fixed	5.14E-			5.45E-		0.00	
	Sex	d	01	2.04E-01	8.30E-01	01	2000	3	**
		fixed	-6.44E-			-1.60E-		0.74	
	Age	d	02	-4.29E-01	3.01E-01	01	2000	4	
								<	
		fixed	1.14E-			1.21E-		0.00	
	Population	d	06	5.69E-07	1.69E-06	06	2000	05	***
	Sex:Population	fixed	-7.47E-			-8.12E-		0.00	
	n	d	07	-1.26E-06	-1.82E-07	07	2000	6	**
		random							
		do	6.40E-			3.57E-			
	County	m	02	4.53E-08	1.69E-01	02	2194	NA	
		residual	9.16E-			9.12E-			
Residuals		dual	01	7.58E-01	1.08E+00	01	2059	NA	
Profile PC2		fixed	2.66E+0			2.60E+0		0.00	
Feeder Abundance DIC = 427.989	Intercept	d	1	1.00E+01	4.44E+01	1	2630	4	**
		fixed	-1.40E-			-1.31E-		0.00	
	Year	d	02	-2.31E-02	-5.28E-03	02	2606	4	**
		fixed	4.11E-			5.07E-		0.06	
	Sex	d	01	-2.66E-02	8.52E-01	01	2000	9	.
		fixed	-2.57E-			-2.75E-		0.26	
	Age	d	01	-6.78E-01	2.04E-01	01	2040	1	

								<
		Feeder	fixe	4.22E-		3.85E-	0.00	
		Abundance	d	02	2.06E-02	6.56E-02	02	1794 05 ***
		Sex:Feeder	fixe	-1.23E-		-1.77E-	0.23	
		Abundance	d	02	-3.19E-02	8.53E-03	02	2000 2
			ran					
			do	6.00E-		2.65E-		
		County	m	02	2.91E-07	2.13E-01	04	2000 NA
			resi	9.23E-		8.63E-		
		Residuals	dual	01	7.15E-01	1.16E+00	01	2000 NA
Profile PC2								
Eucalyptus			fixe	2.72E+0		3.67E+0	0.55	
Abundance		Intercept	d	0	-8.07E+00	1.14E+01	0	2000 3
			fixe	-1.54E-		-2.05E-	0.51	
DIC = 654.6988		Year	d	03	-6.22E-03	3.86E-03	03	2133 5
			fixe	2.84E-		3.75E-		
		Sex	d	01	-2.22E-01	7.43E-01	01	2000 0.27
			fixe	-1.55E-		-2.12E-		
		Age	d	01	-5.57E-01	2.44E-01	01	2000 0.46
		Eucalyptus	fixe	2.32E-		2.34E-	0.27	
		Abundance	d	03	-2.06E-03	6.24E-03	03	2000 3
		Sex:Eucalyptu	fixe	-1.41E-		3.82E-	0.95	
		s Abundance	d	04	-4.41E-03	4.45E-03	04	2123 2
			ran					
			do	7.00E-		1.30E-		
		County	m	02	1.72E-08	2.15E-01	03	2000 NA
			resi	9.73E-		9.33E-		
		Residuals	dual	01	8.03E-01	1.16E+00	01	1886 NA
Profile PC2								
Climate			fixe	1.77E+0		1.78E+0	0.69	
		Intercept	d	0	-1.05E+01	6.90E+00	0	2000 2
			fixe	8.17E-		1.02E-	0.72	
DIC = 686.1844		Year	d	04	-3.44E-03	5.58E-03	03	2000 2
			fixe	2.40E-		2.12E-	0.08	
		Sex	d	01	-4.43E-02	5.15E-01	01	1919 9

<i>Profile PC2 Base Model</i> <i>DIC = 783.6967</i>		fixe	-7.68E-			-1.43E-		
	Age	d	02	-4.58E-01	3.20E-01	01	2000	0.69
	Mean	fixe	-2.09E-			-8.39E-		0.91
	Temperature	d	03	-3.62E-02	3.65E-02	03	2000	1
	Monthly	fixe	1.54E-			1.75E-		0.17
	Precipitation	d	03	-5.42E-04	3.74E-03	03	2000	3
		ran						
		do	1.04E-			5.67E-		
	County	m	01	1.63E-05	2.56E-01	02	2000	NA
		resi	9.43E-			9.31E-		
	Residuals	dual	01	7.65E-01	1.12E+00	01	2000	NA
			-			-		
		fixe	6.76E+0			9.01E+0		0.28
	Intercept	d	0	-2.03E+01	4.77E+00	0	2153	7
		fixe	3.42E-			4.60E-		0.29
	Year	d	03	-2.60E-03	1.04E-02	03	2155	5
		fixe	1.04E+0			1.29E+0		0.14
	Sex	d	1	-3.86E+00	2.54E+01	1	1883	1
		fixe	-1.43E-			-1.46E-		0.45
	Age	d	01	-5.23E-01	2.26E-01	01	2136	6
		fixe	-5.23E-			-6.62E-		0.15
	Year:Sex	d	03	-1.32E-02	2.00E-03	03	1888	2
		ran						
		do	5.24E-			1.06E-		
	County	m	02	2.46E-07	1.45E-01	03	1755	NA
		resi	9.84E-			9.80E-		
	Residuals	dual	01	8.21E-01	1.16E+00	01	2000	NA

Table S13. Bill dorsal area MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

				Lower 95%	Upper 95%		Effective	pM	
	Variable	Effect	Posterior Mean	Credible Interval	Credible Interval	Posterior Mode	Sample Size	CM	Significance
Dorsal Area Population DIC = 704.6589		fixed	13.5474			13.3194		0.00	
	Intercept	d	988	3.89225692	23.9592894	2	2327	7	**
			-			-			
		fixed	0.00688			0.00732		0.00	
	Year	d	72	-0.0122988	-0.0018798	92	2327	8	**
			-			-			
		fixed	0.34947			0.41621		0.01	
	Sex	d	51	-0.6067723	-0.0765742	53	2283	1	*
		fixed	0.11254			0.09567		0.47	
	Age	d	869	-0.2082968	0.43476819	167	2000	4	
		fixed	5.11E-			4.80E-		0.05	
	Population	d	07	-4.45E-08	1.03E-06	07	2000	8	.
	Sex:Population	fixed	-2.69E-			-2.95E-			
	n	d	07	-7.62E-07	1.96E-07	07	2094	0.29	
		random							
		do	0.21008			0.14744			
Dorsal Area Feeder Abundance DIC = 393.3847	County	m	594	0.05956855	0.38289285	41	2000	NA	
		residual	0.67023			0.65773			
	Residuals	dual	058	0.5518459	0.77745673	36	2000	NA	
		fixed	28.1628			27.3371		0.00	
	Intercept	d	683	8.49976225	46.188745	9	2000	1	***
			-			-			
		fixed	0.01452			0.01578		0.00	
	Year	d	13	-0.0237246	-0.0043012	43	2000	1	***
			-			-			
		fixed	0.05557			0.08222		0.78	
	Sex	d	77	-0.4965811	0.35591227	4	2000	4	
		fixed	0.17993			0.14220		0.33	
	Age	d	702	-0.2056189	0.56402166	42	2000	9	

	Feeder	fixe	0.02772			0.03291		0.02	
	Abundance	d	145	0.00370019	0.05107794	985	2000	5	*
			-						
	Sex:Feeder	fixe	0.00626			-		0.51	
	Abundance	d	62	-0.0230081	0.01332875	0.00692	2000	1	
		ran							
		do	0.29226			0.15907			
	County	m	115	0.03744662	0.69825034	03	1737	NA	
		resi	0.71623			0.66275			
	Residuals	dual	306	0.54221718	0.87128783	46	2257	NA	
Dorsal Area									
Eucalyptus		fixe	13.0561			13.6983		0.01	
Abundance	Intercept	d	371	2.53402426	24.791706	5	2000	7	*
			-			-			
		fixe	0.00663			0.00712			
DIC = 616.7642	Year	d	57	-0.0128215	-0.0012636	39	1975	0.02	*
			-			-			
		fixe	0.33744			0.31393		0.07	
	Sex	d	79	-0.7389602	0.01798157	43	2001	8	.
		fixe	0.16219			0.10494		0.37	
	Age	d	633	-0.1794593	0.5077229	02	2000	3	
	Eucalyptus	fixe	0.00344			0.00372		0.13	
	Abundance	d	376	-0.0014563	0.00801257	423	2000	7	
			-			-			
	Sex:Eucalyptu	fixe	0.00097			0.00132		0.60	
s Abundance	d	51	-0.0047915	0.00262731	53	2000	7		
		ran							
		do	0.28777			0.21597			
County	m	754	0.05982074	0.57990536	19	2000	NA		
	resi	0.69667			0.69216				
Residuals	dual	937	0.58103305	0.83520027	83	2000	NA		
Dorsal Area									
Climate		fixe	6.95167			5.73118			
	Intercept	d	002	-0.4655117	14.5540701	4	2000	0.07	.

DIC = 652.7144			-			-			
		fixe	0.00383			0.00317		0.05	
	Year	d	57	-0.0078685	-5.94E-05	38	2000	8	.
			-			-		<	
		fixe	0.42039			0.44950		0.00	
	Sex	d	74	-0.6853421	-0.1758392	5	2000	05	***
		fixe	0.07457			0.09277		0.63	
	Age	d	233	-0.2379141	0.38695359	903	2180	6	
	Mean	fixe	0.04503			0.04458		0.00	
	Temperature	d	17	0.01463361	0.07453917	354	2000	3	**
	Monthly	fixe	0.00247			0.00250		0.00	
	Precipitation	d	036	0.0005557	0.00412393	876	1846	7	**
		ran							
		do	0.19214			0.13809			
	County	m	55	0.04887363	0.37734128	26	2000	NA	
		resi	0.69023			0.68660			
	Residuals	dual	153	0.57977024	0.82665937	92	1876	NA	
Dorsal Area Base Model DIC = 723.0348		fixe	6.29552			6.64278		0.27	
	Intercept	d	907	-4.6232541	17.1112429	8	2149	2	
			-			-			
		fixe	0.00309			0.00262		0.29	
	Year	d	12	-0.0085803	0.00267521	98	2144	3	
			-			-			
		fixe	0.04848			2.93879		0.98	
	Sex	d	38	-12.247688	11.3743027	3	2000	5	
		fixe	0.12013			0.16301		0.46	
	Age	d	583	-0.2215712	0.40221286	83	2000	8	
			-						
		fixe	0.00019			0.00051		0.96	
	Year:Sex	d	06	-0.0060477	0.00616232	706	2000	3	
		ran							
		do	0.21752			0.16070			
	County	m	317	0.05922874	0.41334673	03	1752	NA	
		resi	0.66071			0.66321			
	Residuals	dual	307	0.54233559	0.76707456	22	2000	NA	

Table S14. Dorsal PC1 MCMCglmm output summaries. A ‘.’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1. Principal components of bill shape should be unaffected by body size as the geomorphometric methods that generated these PC values remove this effect, so body size was not included as a covariate.

				Lower 95%	Upper 95%		Effective	pM	
		Effect	Posterior	Credible	Credible	Posterior	Sample	CM	Signifi
		Variable	Mean	Interval	Interval	r Mode	Size	C	cance
Dorsal PC1 Population DIC = 714.7124		fixed	6.29E-			2.73E-			
	Intercept	d	01	-8.50E+00	9.40E+00	01	2000	0.89	
		fixed	-3.17E-			1.14E-		0.89	
	Year	d	04	-4.92E-03	4.41E-03	03	2000	3	
		fixed	-1.97E-			-1.80E-		0.15	
	Sex	d	01	-4.78E-01	7.58E-02	01	2000	2	
		fixed	2.34E-			2.34E-		0.12	
	Age	d	01	-8.35E-02	5.25E-01	01	2000	5	
		fixed	-2.46E-			-2.75E-		0.37	
	Population	d	07	-7.94E-07	2.96E-07	07	2000	8	
	Sex:Population	fixed	5.24E-			5.40E-		0.04	
	n	d	07	2.61E-08	1.06E-06	07	2000	5	*
		ran							
	do		1.88E-			1.62E-			
	County	m	02	3.65E-09	6.96E-02	04	1843	NA	
		resi	7.46E-			7.24E-			
	Residuals	dual	01	6.28E-01	8.84E-01	01	2000	NA	
Dorsal PC1 Feeder Abundance DIC = 385.6338		fixed	1.98E+0	-1.56E+01	1.24E+01	3.06E+0	2000	0.79	
	Intercept	d	0			0		7	
		fixed	9.68E-			6.08E-		0.80	
	Year	d	04	-6.45E-03	8.20E-03	05	2000	3	
		fixed	-5.44E-			-9.18E-		0.98	
	Sex	d	03	-4.03E-01	3.87E-01	03	2095	9	
		fixed	2.36E-			2.81E-		0.24	
	Age	d	01	-1.36E-01	6.43E-01	01	2118		
	Feeder	fixed	-6.42E-			-7.28E-		0.52	
	Abundance	d	03	-2.53E-02	1.55E-02	03	2000	9	

<i>Dorsal PCI </i> <i>Eucalyptus</i> <i>Abundance</i> <i>DIC = 618.3266</i>	Sex:Feeder	fixe	1.50E-			1.45E-	0.10
	Abundance	d	02	-2.96E-03	3.35E-02	02	2000 2
		ran					
		do	1.06E-	1.19E-10	1.23E-01	2.74E-	1986 NA
	County	m	03			02	
		resi	6.71E-			7.27E-	
	Residuals	dual	01	5.62E-01	0.9035922	01	2000 NA
	<i>Dorsal PCI </i>		-			-	
	<i>Eucalyptus</i>	fixe	2.66E+0			3.28E+0	0.45
	<i>Abundance</i>	Intercept	d	0	-9.67E+00	5.02E+00 0	2000 2
		fixe	1.27E-			1.44E-	0.48
		Year	d	03	-2.68E-03	4.88E-03 03	2000 7
		fixe	8.12E-			6.44E-	0.67
		Sex	d	02	-2.87E-01	4.87E-01 02	2000 7
		fixe	1.69E-			2.20E-	0.32
		Age	d	01	-1.85E-01	5.04E-01 01	2000 5
	<i>Eucalyptus</i>	fixe	1.46E-			1.31E-	0.40
	<i>Abundance</i>	d	03	-1.80E-03	4.94E-03	03	2289 4
	Sex: <i>Eucalyptu</i>	fixe	-3.74E-			-3.91E-	0.86
	s <i>Abundance</i>	d	04	-4.08E-03	3.44E-03	04	2202 5
		ran					
		do	2.38E-			3.17E-	
	County	m	02	3.78E-07	8.84E-02	04	1835 NA
		resi	7.43E-			7.53E-	
	Residuals	dual	01	6.09E-01	8.71E-01	01	2000 NA
	<i>Dorsal PCI </i>		-			-	
	<i>Climate</i>	fixe	2.48E+0			3.27E+0	
		Intercept	d	0	-8.92E+00	4.49E+00 0	2000 0.45
		fixe	1.22E-			1.79E-	0.47
		Year	d	03	-2.18E-03	4.62E-03 03	2000 4
		fixe	-4.31E-			-4.49E-	0.71
		Sex	d	02	-2.99E-01	1.96E-01 02	2000 1
		fixe	2.20E-			1.83E-	0.20
		Age	d	01	-1.14E-01	5.54E-01 01	2000 4

<i>Dorsal PC1 Base Model</i> <i>DIC = 740.399</i>	Mean	fixe	3.46E-			5.77E-	0.81	
	Temperature	d	03	-2.53E-02	3.51E-02	03	2000	8
	Monthly	fixe	6.36E-			4.64E-		0.46
	Precipitation	d	04	-1.10E-03	2.39E-03	04	2391	4
		ran						
		do	1.17E-			1.11E-		
	County	m	02	2.72E-09	4.83E-02	04	2000	NA
		resi	7.58E-			7.34E-		
	Residuals	dual	01	6.33E-01	9.02E-01	01	2148	NA
		fixe	1.07E+0			9.93E+0		0.04
	Intercept	d	1	6.13E-02	2.11E+01	0	2000	3 *
		fixe	-5.55E-			-6.03E-		0.04
	Year	d	03	-1.10E-02	-8.42E-05	03	2000	3 *
		-				-		
		fixe	1.92E+0			1.84E+0		0.00
	Sex	d	1	-3.10E+01	-6.45E+00	1	2000	4 **
		fixe	2.73E-			1.71E-		0.08
	Age	d	01	-2.35E-02	5.85E-01	01	2000	3 .
		fixe	9.89E-			9.40E-		0.00
	Year:Sex	d	03	3.47E-03	1.62E-02	03	2000	4 **
		ran						
		do	2.63E-			9.51E-		
	County	m	02	2.29E-09	9.32E-02	04	2000	NA
		resi	7.53E-			7.56E-		
	Residuals	dual	01	6.28E-01	8.82E-01	01	2000	NA

Table S15. Dorsal PC2 MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1. Principal components of bill shape should be unaffected by body size as the geomorphometric methods that generated these PC values remove this effect, so body size was not included as a covariate.

			Lower 95%	Upper 95%		Effective	pM	
			Credible	Credible	Posterior	Sample	CM	Signifi
			Interval	Interval	r Mode	Size	C	cance
Dorsal PC2 Population DIC = 785.3938		fixe	2.22E+0		2.36E+0			
	Intercept	d	0	-1.42E+01	8.75E+00	0	2000	0.69
		fixe	1.28E-		1.80E-			
	Year	d	03	-4.43E-03	7.47E-03	03	2000	0.66
		fixe	-3.88E-		-3.64E-			0.02
	Sex	d	01	-6.88E-01	-7.54E-02	01	1671	3 *
		fixe	-1.65E-		-1.17E-			0.36
	Age	d	01	-5.25E-01	1.79E-01	01	2000	2
		fixe	-5.30E-		-6.81E-			0.09
	Population	d	07	-1.18E-06	5.83E-08	07	2143	1 .
	Sex:Population	fixe	9.44E-		9.58E-			0.00
	n	d	07	3.55E-07	1.49E-06	07	2000	4 **
		ran						
		do	6.72E-		4.51E-			
	County	m	02	2.89E-06	1.69E-01	02	2134	NA
	resi	9.54E-		9.51E-				
Residuals	dual	01	7.90E-01	1.12E+00	01	2000	NA	
Dorsal PC2 Feeder Abundance DIC = 467.272		fixe	1.03E+0		1.79E+0			0.93
	Intercept	d	0	-1.81E+01	2.01E+01	0	2000	4
		fixe	-6.04E-		-1.87E-			0.91
	Year	d	04	-1.05E-02	9.41E-03	03	2000	6
		fixe	-2.89E-		-2.03E-			0.26
	Sex	d	01	-8.18E-01	2.13E-01	01	1879	5
		fixe	-1.98E-		-1.77E-			0.43
	Age	d	01	-6.95E-01	2.75E-01	01	2000	7

<i>Dorsal PC2 </i> <i>Eucalyptus</i> <i>Abundance</i> <i>DIC = 694.8845</i>	Feeder	fixe	6.92E-			8.39E-	0.63	
	Abundance	d	03	-1.98E-02	3.24E-02	03	2000	3
	Sex:Feeder	fixe	2.13E-			2.58E-		0.07
	Abundance	d	02	-1.12E-03	4.66E-02	02	2000	7
		ran						
		do	4.43E-			6.64E-		
	County	m	02	9.59E-13	1.82E-01	04	2000	NA
		resi	1.26E+0			1.21E+0		
	Residuals	dual	0	9.74E-01	1.55E+00	0	2000	NA
<i>Dorsal PC2 </i> <i>Climate</i> <i>DIC = 736.7223</i>		fixe	8.55E+0			7.82E+0	0.04	
	Intercept	d	0	-1.71E+01	-2.08E-01	0	1892	8 *
		fixe	4.53E-			5.04E-		0.04
	Year	d	03	2.91E-04	8.99E-03	03	1876	7 *
		fixe	-1.09E-			-1.23E-		0.50
	Sex	d	01	-4.32E-01	1.69E-01	01	1680	7

Dorsal PC2 Base Model DIC = 812.3601		fixe	-2.26E-			-1.97E-	0.26
	Age	d	01	-6.28E-01	1.68E-01	01	2000 9
	Mean	fixe	-8.29E-			-4.34E-	0.66
	Temperature	d	03	-4.56E-02	2.91E-02	03	2208 2
	Monthly	fixe	-3.18E-			6.20E-	0.77
	Precipitation	d	04	-2.53E-03	1.64E-03	05	2167 7
		ran					
		do	4.41E-			1.01E-	
	County	m	02	2.91E-08	1.39E-01	03	1816 NA
		resi	1.06E+0			1.05E+0	
	Residuals	dual	0	8.62E-01	1.24E+00	0	2145 NA
		fixe	1.01E+0			1.54E+0	
	Intercept	d	0	-1.05E+01	1.40E+01	0	2000 0.89
		fixe	-4.62E-			-6.01E-	0.90
	Year	d	04	-7.20E-03	5.46E-03	04	2000 2
		-				-	
		fixe	1.15E+0			1.31E+0	0.12
	Sex	d	1	-2.51E+01	2.67E+00	1	2000 5
		fixe	-2.01E-			-2.43E-	0.24
	Age	d	01	-5.45E-01	1.46E-01	01	2000 2
		fixe	5.90E-			6.76E-	0.13
	Year:Sex	d	03	-1.35E-03	1.30E-02	03	2000 2
		ran					
		do	4.95E-			1.11E-	
	County	m	02	6.27E-08	1.33E-01	03	2000 NA
		resi	9.66E-			9.13E-	
	Residuals	dual	01	8.03E-01	1.13E+00	01	2000 NA

Table S16. Body length MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

				Lower 95%	Upper 95%		Effective	pM	
		Effect	Posterior	Credible	Credible	Posterior	Sample	CM	Signifi
Variable		ct	r Mean	Interval	Interval	r Mode	Size	C	cance
Body Length Population DIC = 737.8163		fixe	-1.45E-			-1.17E-		0.97	
	(Intercept)	d	01	-1.27E+01	1.23E+01	01	2000	8	
		fixe	5.12E-			1.84E-		0.98	
	Year	d	05	-6.30E-03	6.70E-03	04	2000	7	
		fixe	5.77E-			2.15E-		0.72	
	Sex	d	02	-2.56E-01	3.56E-01	03	2000	5	
		fixe	1.02E-			5.06E-		0.56	
	Age	d	01	-2.50E-01	4.43E-01	02	1868	4	
		fixe	3.06E-			3.99E-		0.32	
	Population	d	07	-3.19E-07	8.93E-07	07	1803	6	
	Sex:Population	fixe	-3.21E-			-4.24E-		0.26	
	n	d	07	-9.39E-07	2.08E-07	07	1845	2	
		ran							
		do	2.65E-			5.88E-			
	County	m	02	3.65E-09	1.05E-01	04	2075	NA	
		ran							
		do	2.04E-			1.34E-			
	Preparators	m	01	3.82E-02	3.85E-01	01	2000	NA	
		resi	7.62E-			7.55E-			
	Residuals	dual	01	6.16E-01	9.07E-01	01	2000	NA	
Body Length Feeder Abundance DIC = 417.5462		fixe	8.86E+0			1.49E+0		0.38	
	(Intercept)	d	0	-1.28E+01	2.82E+01	1	1754	1	
		fixe	-4.49E-			-7.43E-		0.39	
	Year	d	03	-1.43E-02	6.97E-03	03	1750	6	
		fixe	2.39E-			3.24E-		0.93	
	Sex	d	02	-4.57E-01	5.12E-01	02	1991	4	
		fixe	-1.74E-			-1.33E-		0.45	
	Age	d	01	-6.18E-01	2.70E-01	01	2000	3	
	Feeder	fixe	9.93E-			1.14E-		0.43	
	Abundance	d	03	-1.52E-02	3.50E-02	02	2000	8	

		Sex:Feeder	fixe	-7.45E-		-7.57E-		0.46
	Abundance	d	03	-2.80E-02	1.22E-02	03	2000	5
		ran						
		do		8.41E-		2.30E-		
	County	m	02	1.24E-08	3.02E-01	03	2000	NA
		ran						
		do		8.90E-		1.44E-		
	Preparators	m	02	1.15E-06	2.47E-01	03	2159	NA
		resi		8.55E-		8.43E-		
	Residuals	dual	01	6.42E-01	1.07E+00	01	2000	NA
<i>Body Length </i>				-				
	<i>Eucalyptus</i>		fixe	2.08E+0		6.21E-		0.73
	Abundance	(Intercept)	d	0	-1.29E+01	9.90E+00	01	2492 8
			fixe	1.07E-		1.43E-		0.74
	Year	d	03	-5.15E-03	6.60E-03	03	2495	4
			fixe	4.85E-		8.02E-		0.80
	Sex	d	02	-3.62E-01	4.70E-01	03	2082	8
			fixe	8.48E-		-3.18E-		0.97
	Age	d	03	-3.83E-01	3.76E-01	02	2000	8
	<i>Eucalyptus</i>		fixe	1.78E-		2.21E-		0.37
<i>DIC = 652.9939</i>	Abundance	d	03	-2.27E-03	5.90E-03	03	1849	8
	Sex: <i>Eucalyptu</i>		fixe	-1.84E-		-2.17E-		0.36
	s Abundance	d	03	-5.61E-03	2.33E-03	03	1879	3
		ran						
		do		4.50E-		1.68E-		
	County	m	02	6.47E-08	1.63E-01	03	2000	NA
		ran						
		do		2.08E-		1.85E-		
	Preparators	m	01	3.34E-02	4.16E-01	01	2000	NA
		resi		7.61E-		7.56E-		
	Residuals	dual	01	6.07E-01	9.19E-01	01	1939	NA
				-		-		
	<i>Body Length </i>		fixe	1.51E+0		1.49E+0		0.77
	<i>Climate</i>	(Intercept)	d	0	-1.10E+01	8.55E+00	0	2000 3

DIC = 669.3136		fixe	6.02E-			1.87E-	0.82
	Year	d	04	-4.67E-03	5.43E-03	03	2000 5
		fixe	-5.62E-			8.06E-	
	Sex	d	03	-3.09E-01	2.53E-01	03	1782 0.97
		fixe	9.38E-			1.32E-	0.61
	Age	d	02	-2.59E-01	4.54E-01	01	2000 4
	Mean	fixe	1.97E-			2.06E-	0.23
	Temperature	d	02	-1.62E-02	5.07E-02	02	1875 3
	Monthly	fixe	1.33E-			9.12E-	0.18
	Precipitation	d	03	-5.56E-04	3.29E-03	04	2000 1
		ran					
		do	2.67E-			7.12E-	
	County	m	02	4.51E-09	1.01E-01	04	2000 NA
		ran					
		do	1.82E-			1.16E-	
	Preparators	m	01	3.51E-02	3.64E-01	01	2000 NA
		resi	7.68E-			7.56E-	
	Residuals	dual	01	6.24E-01	9.18E-01	01	2000 NA
Body Length Base Model DIC = 760.9732			-			-	
		fixe	2.56E+0			3.90E+0	
	(Intercept)	d	0	-1.66E+01	9.95E+00	0	2000 0.7
		fixe	1.33E-			6.30E-	0.69
	Year	d	03	-5.19E-03	8.50E-03	04	2000 8
		fixe	2.35E+0			1.92E+0	0.73
	Sex	d	0	-1.01E+01	1.59E+01	0	2000 9
		fixe	8.68E-			5.36E-	
	Age	d	02	-2.39E-01	4.48E-01	02	2000 0.62
		fixe	-1.24E-			-9.00E-	0.73
	Year:Sex	d	03	-7.93E-03	5.51E-03	04	2000 3
		ran					
		do	3.16E-			2.80E-	
	County	m	02	7.24E-12	1.18E-01	04	2000 NA
		ran					
		do	1.98E-			1.45E-	
	Preparators	m	01	5.37E-02	3.91E-01	01	2000 NA

		resi	7.59E-			7.34E-		
	Residuals	dual	01	6.27E-01	9.20E-01	01	2000	NA

Table S17. Wing chord MCMCglmm output summaries. A ‘.’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

				Lower 95%	Upper 95%		Effective	pM	
		Effect	Posterior	Credible	Credible	Posterior	Sample	CM	Signifi
		Variable	Mean	Interval	Interval	r Mode	Size	C	cance
Wing Chord Population DIC = 790.5715		fixe	8.31E+0			7.44E+0		0.10	
	Intercept	d	0	-1.20E+00	1.82E+01	0	2000	1	
		fixe	-4.23E-			-4.20E-		0.10	
	Year	d	03	-9.43E-03	6.39E-04	03	2000	8	
		fixe	-3.24E-			-3.48E-		0.02	
	Sex	d	01	-5.94E-01	-2.70E-02	01	2000	8	*
		fixe	2.29E-			2.57E-		0.20	
	Age	d	01	-9.74E-02	5.89E-01	01	2000	6	
		fixe	-1.05E-			-8.23E-		0.66	
	Population	d	07	-5.75E-07	3.40E-07	08	2000	1	
	Sex:Population	fixe	4.23E-			4.65E-		0.04	
	n	d	07	5.68E-09	8.29E-07	07	2140	5	*
		ran							
	do		3.89E-			3.84E-			
	County	m	02	8.10E-09	1.42E-01	04	2000	NA	
		resi	8.94E-			8.73E-			
	Residuals	dual	01	7.49E-01	1.05E+00	01	2000	NA	
Wing Chord Feeder Abundance DIC = 429.9105		fixe	4.46E+0			4.16E+0		0.59	
	Intercept	d	0	-1.27E+01	2.25E+01	0	2000	9	
		fixe	-2.32E-			-2.28E-		0.59	
	Year	d	03	-1.15E-02	6.63E-03	03	2000	4	
		fixe	-1.64E-			-9.83E-		0.52	
	Sex	d	01	-6.60E-01	3.24E-01	02	1653	5	
		fixe	3.21E-			2.46E-		0.21	
	Age	d	01	-2.21E-01	7.99E-01	01	2000	6	
	Feeder	fixe	-5.77E-			1.24E-		0.96	
	Abundance	d	04	-2.28E-02	2.20E-02	03	2000	4	
	Sex:Feeder	fixe	1.01E-			1.13E-		0.29	
	Abundance	d	02	-8.35E-03	2.98E-02	02	2000	1	

Wing Chord Eucalyptus Abundance DIC = 688.6246		ran					
		do	9.86E-		1.71E-		
	County	m	02	5.81E-09	3.27E-01	03	2000 NA
		resi	1.13E+0		1.05E+0		
	Residuals	dual	0	8.71E-01	1.41E+00	0	1840 NA
		fixe	2.86E+0		2.08E+0		0.46
	Intercept	d	0	-4.90E+00	1.03E+01	0	1876 1
		fixe	-1.41E-		-1.55E-		0.48
	Year	d	03	-5.20E-03	2.77E-03	03	2000 1
		fixe	-3.80E-		-4.02E-		
	Sex	d	01	-8.26E-01	2.08E-02	01	2000 0.08
		fixe	3.15E-		3.70E-		0.11
	Age	d	01	-6.11E-02	7.09E-01	01	1842 7
	Eucalyptus	fixe	-2.53E-		-3.58E-		0.91
	Abundance	d	04	-4.19E-03	3.87E-03	04	2161 2
	Sex:Eucalyptu	fixe	2.49E-		1.53E-		
	s Abundance	d	03	-1.61E-03	6.46E-03	03	2000 0.24
		ran					
		do	7.13E-		1.79E-		
Wing Chord Climate DIC = 631.6422	County	m	02	1.73E-08	2.56E-01	03	2000 NA
		resi	9.39E-		9.25E-		
	Residuals	dual	01	7.76E-01	1.11E+00	01	2000 NA
		fixe	4.43E+0		3.78E+0		0.13
	Intercept	d	0	-1.06E+00	1.04E+01	0	2000 5
		fixe	-2.18E-		-1.86E-		0.14
	Year	d	03	-5.22E-03	6.01E-04	03	2000 6
		fixe	-2.00E-		-2.33E-		0.08
	Sex	d	01	-4.25E-01	3.16E-02	01	2146 9
		fixe	3.25E-		4.04E-		0.05
	Age	d	01	8.03E-03	6.55E-01	01	2000 1
	Mean	fixe	-1.01E-		-4.20E-		0.46
	Temperature	d	02	-3.80E-02	1.60E-02	03	1771 9
	Monthly	fixe	2.14E-		-2.06E-		0.79
	Precipitation	d	04	-1.68E-03	1.82E-03	04	2000 7

<i>Wing Chord Base</i> <i>Model</i> <i>DIC = 809.7319</i>		ran					
		do	5.12E-		7.71E-		
	County	m	02	4.34E-08	1.74E-01	04	2000 NA
		resi	6.60E-		6.68E-		
	Residuals	dual	01	5.34E-01	7.79E-01	01	2000 NA
		fixe	9.77E+0		1.28E+0		0.05
	Intercept	d	0	-3.32E-01	2.02E+01	1	1857 9
		fixe	-5.01E-		-5.31E-		0.06
	Year	d	03	-1.04E-02	1.54E-04	03	1861 3 .
			-		-		
		fixe	1.01E+0		9.15E+0		0.09
	Sex	d	1	-2.31E+01	1.57E+00	0	2680 7 .
		fixe	2.38E-		2.35E-		0.17
	Age	d	01	-1.13E-01	5.62E-01	01	2000 8 .
		fixe	5.17E-		4.61E-		0.10
	Year:Sex	d	03	-1.57E-03	1.12E-02	03	2682 1
		ran					
		do	3.94E-		8.25E-		
	County	m	02	3.15E-08	1.38E-01	04	2000 NA
		resi	8.79E-		8.56E-		
	Residuals	dual	01	7.32E-01	1.02E+00	01	2000 NA

Table S18. Weight MCMCglmm output summaries. A ‘:’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

			Lower 95%		Upper 95%		Effective	pM			
			Effe	Posterio	Credible	Credible	Posterio	Sample	CM	Signifi	
Variable			ct	r Mean	Interval	Interval	r Mode	Size	C	cance	
Weight			fixe	3.28E+0			3.43E+0		0.74		
Population			(Intercept)	d	0	-1.59E+01	2.18E+01	0	2034	9	
DIC = 232.8237			fixe	-1.84E-			-2.35E-				
			Year	d	03	-1.13E-02	8.27E-03	03	2282	0.72	
			fixe	7.24E-			6.25E-		0.00		
			Sex	d	01	2.47E-01	1.24E+00	01	2000	5	**
			fixe	-5.16E-			-4.73E-				
			Age	d	01	-9.31E-01	-3.32E-02	01	2000	0.03	*
			fixe	1.34E-			9.01E-		0.64		
			Population	d	07	-4.53E-07	6.92E-07	08	2000	6	
			fixe	-1.73E-			1.13E-		0.98		
			Seasonality	d	05	-1.75E-03	1.67E-03	04	2000	9	
			fixe	-1.48E-			-1.15E-		0.54		
			Sex:Population	d	07	-6.14E-07	3.36E-07	07	2000	8	
			ran								
			do	1.83E-			5.17E-				
			County	m	01	4.48E-06	5.68E-01	03	2000	NA	
resi	6.61E-			6.65E-							
Residuals	dual	01	4.57E-01	8.80E-01	01	2000	NA				
Weight Feeder			fixe	9.06E-			1.95E+0				
Abundance			(Intercept)	d	01	-1.87E+01	2.23E+01	0	2000	0.94	
DIC = 168.6601			fixe	-5.20E-			-5.90E-		0.93		
			Year	d	04	-1.05E-02	1.06E-02	04	2232	3	
			fixe	-1.01E-			7.93E-		0.97		
			Sex	d	02	-6.91E-01	6.36E-01	02	2000	3	
			fixe	-5.72E-			-5.77E-		0.01		
			Age	d	01	-1.06E+00	-9.39E-02	01	2000	5	*
			Feeder	fixe	-8.58E-		-4.32E-		0.90		
			Abundance	d	04	-2.07E-02	2.18E-02	03	2000	5	

Weight Eucalyptus Abundance DIC = 194.4097		fixe	-4.08E-			-6.64E-		0.64	
	Seasonality	d	04	-2.13E-03	1.29E-03	04	2000	3	
	Sex:Feeder	fixe	1.42E-			1.21E-		0.14	
	Abundance	d	02	-5.16E-03	3.19E-02	02	2000	7	
		ran							
		do	9.90E-			1.63E-			
	County	m	02	1.64E-08	4.12E-01	03	2000	NA	
		resi	5.53E-			4.97E-			
	Residuals	dual	01	3.69E-01	7.70E-01	01	2000	NA	
		fixe	2.74E+0			3.41E-		0.75	
	(Intercept)	d	0	-1.26E+01	1.97E+01	01	2000	3	
		fixe	-1.69E-			-2.66E-		0.70	
	Year	d	03	-1.04E-02	6.16E-03	04	2000	4	
		fixe	9.06E-			8.95E-		0.00	
	Sex	d	01	3.08E-01	1.55E+00	01	2000	3	**
		fixe	-4.82E-			-5.03E-		0.04	
	Age	d	01	-9.49E-01	3.41E-03	01	2000	2	*
	Eucalyptus	fixe	6.32E-			7.21E-		0.10	
	Abundance	d	03	-1.75E-03	1.37E-02	03	2000	5	
		fixe	-7.43E-			-7.67E-		0.39	
	Seasonality	d	04	-2.56E-03	8.78E-04	04	2000	7	
	Sex:Eucalyptu	fixe	-4.74E-			-5.65E-		0.17	
	s Abundance	d	03	-1.19E-02	1.47E-03	03	2000	6	
		ran							
		do	1.22E-			2.24E-			
	County	m	01	6.76E-08	4.67E-01	03	1843	NA	
		resi	5.73E-			5.53E-			
	Residuals	dual	01	3.95E-01	7.65E-01	01	2000	NA	
Weight Climate DIC = 227.3971		fixe	2.75E+0			1.26E+0		0.63	
	(Intercept)	d	0	-9.37E+00	1.42E+01	0	2000	5	
		fixe	-1.42E-			-1.91E-		0.62	
	Year	d	03	-7.34E-03	4.66E-03	03	2000	5	
		fixe	5.99E-			6.23E-		0.00	
	Sex	d	01	2.16E-01	9.35E-01	01	2000	1	***

<i>Weight Base</i> <i>Model</i> <i>DIC = 265.2658</i>		fixe	-3.98E-			-4.12E-		0.10
	Age	d	01	-8.62E-01	9.51E-02	01	1824	4
	Mean	fixe	-1.95E-			-2.49E-		0.43
	Temperature	d	02	-6.80E-02	3.27E-02	02	2000	2
	Monthly	fixe	1.87E-			1.75E-		0.37
	Precipitation	d	03	-2.13E-03	6.39E-03	03	2000	3
		ran						
		do	1.27E-			5.43E-		
	County	m	01	3.46E-11	4.48E-01	04	2000	NA
		resi	6.54E-			6.23E-		
	Residuals	dual	01	4.57E-01	8.71E-01	01	2000	NA
		fixe	2.20E+0			5.32E+0		0.82
	(Intercept)	d	0	-1.40E+01	1.90E+01	0	2000	6
		fixe	-1.25E-			6.57E-		0.79
	Year	d	03	-9.47E-03	7.39E-03	04	2000	6
		fixe	5.97E+0			6.36E+0		0.55
	Sex	d	0	-1.28E+01	2.70E+01	0	2000	1
		fixe	-4.05E-			-5.38E-		0.09
	Age	d	01	-8.69E-01	4.57E-02	01	1840	1
		fixe	-1.02E-			-1.67E-		0.89
	Seasonality	d	04	-1.69E-03	1.60E-03	05	2000	3
		fixe	-2.67E-			-2.73E-		0.59
	Year:Sex	d	03	-1.34E-02	6.76E-03	03	2000	7
		ran						
		do	9.93E-			1.61E-		
	County	m	02	2.06E-08	3.30E-01	03	2256	NA
		resi	7.49E-			7.54E-		
	Residuals	dual	01	5.42E-01	9.79E-01	01	2000	NA

Table S19. Linear model of bird abundance by year model summary; bird abundance is significantly associated with all variables associated with “urbanization”. ‘.’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

		<i>Coefficient</i>	<i>Lower</i>	<i>Upper</i>	<i>P</i>	<i>Significa</i>	
		<i>Estimate (β)</i>	<i>95% CI</i>	<i>95% CI</i>		<i>nance</i>	
<i>Bird Abundance</i> ~ <i>Year</i>	Intercept	-2.52E+01	-2.85E+01	-2.19E+01	2.00E-16	***	
	Year	1.69E-02	1.52E-02	1.86E-02	2.00E-16	***	
	Feeder Abundance	-2.74E-02	-3.71E-02	-1.77E-02	3.51E-08	***	R-squared = 0.3377
	<i>Eucalyptus</i> Abundance	1.38E-03	1.17E-03	1.59E-03	2.00E-16	***	Adjusted R-squared = 0.3367
	Population	5.26E-08	3.98E-08	6.54E-08	9.75E-16	***	
	Latitude	-2.33E-01	-2.48E-01	-2.18E-01	2.00E-16	***	
	Year:Feeder Abundance	3.11E-07	1.77E-07	4.45E-07	5.71E-06	***	

Table S20. Summaries of linear models using significant terms in MCMCglmm models as input. The following models use latitude as a covariate instead of county as a random effect to control for spatial variation. Terms are then dropped iteratively using the step function to converge on the best model. ‘.’ between variables names indicates an interaction effect. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

	<i>Terms</i>	<i>Coefficient Estimate (β)</i>	<i>Lower 95% CI</i>	<i>Upper 95% CI</i>	<i>P</i>	<i>Significance</i>	
<i>Profile PC1</i> ~ Year	Year	1.73E-02	1.04E-02	2.43E-02	2.06E-06	***	
	Sex	3.83E-01	7.54E-02	6.90E-01	1.50E-02	*	R-squared = 0.2656
	Feeder Abundance	3.50E+00	1.70E+00	5.30E+00	1.85E-04	***	Adjusted R-squared = 0.2453
	Year:Feeder Abundance	-1.76E-03	-2.65E-03	-8.62E-04	1.58E-04	***	
<i>Profile PC2</i> ~ Year	Year	-1.08E-02	-2.03E-02	-1.28E-03	2.64E-02	*	
	Sex	3.24E-01	-3.79E-03	6.52E-01	5.27E-02	.	R-squared = 0.1911
	Feeder Abundance	-2.45E+00	-4.35E+00	-5.50E-01	1.19E-02	*	Adjusted R-squared = 0.163
	Latitude	-8.80E-02	-2.02E-01	2.59E-02	1.29E-01		
	Year:Feeder Abundance	1.24E-03	2.89E-04	2.18E-03	1.09E-02	*	
<i>Dorsal PC1</i> ~ Age	Age	2.27E-01	-1.50E-01	6.04E-01	2.36E-01		
	Sex(Female):Feeder Abundance:Latitude	-1.10E-04	-4.38E-04	2.19E-04	5.10E-01		R-squared = 0.04176
	Sex(Male):Feeder Abundance:Latitude	2.74E-04	4.98E-06	5.43E-04	4.60E-02	*	Adjusted R-squared = 0.02179
<i>Dorsal PC2</i> ~ Year	Year	8.04E-03	-4.54E-03	2.06E-02	2.09E-01		
	Sex	1.34E+01	1.46E+01	4.13E+01	3.48E-01		

	<i>Eucalyptus</i> Abundance	1.26E-01	-1.50E-01	4.01E-01	3.69E-01	
	Year:Sex	-1.15E-02	-2.62E-02	3.21E-03	1.25E-01	
	Year: <i>Eucalyptus</i> Abundance	-1.01E-04	-3.00E-04	9.85E-05	3.20E-01	R-squared = 0.1478
	Sex: <i>Eucalyptus</i> Abundance	-3.77E-01	-6.76E-01	-7.76E-02	1.38E-02	Adjusted R-squared = 0.1061
	Sex(Female):Latitude	-6.01E-02	-2.38E-01	1.18E-01	5.08E-01	
	Sex(Male):Latitude	1.82E-01	2.84E-02	3.36E-01	2.05E-02	*
	<i>Eucalyptus</i> Abundance:Latitude	1.82E-03	-2.50E-03	6.14E-03	4.07E-01	
	Year:Sex: <i>Eucalyptus</i> Abundance	3.13E-04	9.27E-05	5.33E-04	5.56E-03	**
	Sex: <i>Eucalyptus</i> Abundance:Latitude	-6.34E-03	-1.15E-02	-1.19E-03	1.61E-02	*

Table S21. ANOVA summary statistics for linear models of culmen length and dorsal area by mean annual temperature. Significance levels: *** = 0.001, ** = 0.01, * = 0.05, . = 0.1.

	<i>Sum Sq</i>		<i>Df</i>		<i>F value</i>		<i>Pr(>F)</i>	
<i>Culmen Length ~ Temperature</i>	Min.	1.90E+01	Min.	1	Min.	4.96E+00	Min.	0.00E+00
	1st Qu.	1.63E+02	1st Qu.	2.5	1st Qu.	2.23E+03	1st Qu.	1.70E-04
	Median	3.07E+02	Median	4	Median	4.46E+03	Median	3.40E-04
	Mean	2.96E+03	Mean	108.3	Mean	4.46E+03	Mean	3.40E-04
	3rd Qu.	4.43E+03	3rd Qu.	162	3rd Qu.	6.69E+03	3rd Qu.	5.10E-04
	Max.	8.55E+03	Max.	320	Max.	8.92E+03	Max.	6.80E-04
					NA's	1	NA's	1
<i>Dorsal Area ~ Temperature</i>	Min.	4.03E+08	Min.	1	Min.	7.87E+00	Min.	0.00E+00
	1st Qu.	2.09E+09	1st Qu.	2.5	1st Qu.	2.35E+02	1st Qu.	1.20E-06
	Median	3.78E+09	Median	4	Median	4.62E+02	Median	2.40E-06
	Mean	5.30E+09	Mean	100	Mean	4.62E+02	Mean	2.40E-06
	3rd Qu.	7.76E+09	3rd Qu.	149.5	3rd Qu.	6.89E+02	3rd Qu.	3.60E-06
	Max.	1.17E+10	Max.	295	Max.	9.17E+02	Max.	4.80E-06
					NA's	1	NA's	1

	<i>Variables</i>	<i>Sum Sq</i>	<i>Df</i>	<i>F value</i>	<i>P</i>	<i>Significance</i>
<i>Culmen Length ~ Temperature</i>	(Intercept)	8.55E+03	1	8.92E+03	< 2.20E-16	***
	Temperature	1.90E+01	4	4.96E+00	6.80E-04	***
	Residuals	3.07E+02	320	NA	NA	NA
<i>Dorsal Area ~ Temperature</i>	(Intercept)	1.17E+10	1	9.17E+02	< 2.20E-16	***
	Temperature	4.03E+08	4	7.87E+00	4.84E-06	***
	Residuals	3.78E+09	295	NA	NA	NA

Chapter Two

Opsin Evolution in the Broad-tailed Hummingbird

Introduction

Most animals use the spectral information in light to forage for food, find mates, and navigate suitable habitat (1). Discriminating spectral information requires at least two receptor types, known as cone cells, which have different spectral sensitivities that allow for comparison of long vs. short wavelengths. For example, the majority of mammals have two types of cone cells tuned to long and short wavelengths, resulting in dichromatic color vision. In contrast, many birds, fish, and reptiles are tetrachromatic and can distinguish a multitude of other colors using at least four cone cell types (2).

Color vision in birds is canonically mediated by the four paralogous, light-sensitive opsins in each cone cell type, which have an evolutionary history characterized by repeated gains and losses (3). Many opsin genes are hypothesized to have been lost due to changes in niche, including adaptations to novel photic pressures or relaxed selection. These losses are found to be increasingly common, despite strong purifying selection acting on opsin coding sequences across birds (4). The loss of opsin genes typically co-occurs with the spectral tuning of cone cells and may increase light sensitivity in a subset of cone cell types (5, 6). Additionally, spectral tuning of one opsin protein via amino acid changes are often followed by complementary tuning of other remaining opsin proteins to optimize color discrimination (6). Complementary spectral tuning can occur via amino acid substitutions or by altering the composition of light-transmitting media in cone cells (6–9).

The enhanced spectral sensitivities of opsins are most salient in animals that produce oil droplets in the inner cone segment. Oil droplets act as long wavelength cutoff filters that shift the peak absorbance of opsins, as in lungfish, reptiles, and birds (10). Tetrachromatic taxa with oil droplets like birds have photoreceptor types paired with a distinctive oil droplet and opsin proteins (11). Together, the opsin protein and chemically distinct oil droplets remove the overlapping sensitivity between receptor types, narrowing their spectral absorbance, and improving color discrimination capabilities. Birds, as a result, have some of the most sophisticated color vision capabilities in the animal kingdom (1).

Ultraviolet Vision

Birds were hypothesized to have trichromatic vision until it was behaviorally demonstrated that hummingbirds could perceive UV light (12, 13). Measurements of transretinal voltages found photopic sensitivity within the UV spectrum at 370 nm, which comported with behavioral data (14). The majority of known avian visual systems consist of four distinct cone cell types that contribute to color vision. Each cone is characterized by an opsin protein that absorbs photons and enters a signaling state of peak sensitivity (λ_{max}) between 310 and 630 nm (15). M-type cone cells typically contain medium-wavelength sensitive (RH2) opsin and are sensitive to middle wavelengths such as green. L-type cells, in contrast, are sensitive to longer wavelengths like reds, and contain long-wavelength sensitive (LWS) opsin. Lastly, there are two types of short wavelength cone cells (S-type). One type absorbs short wavelengths in the blue

spectrum and contains short-wavelength sensitive type 2 (SWS2) opsin. The second type of S-type cell contains short-wavelength sensitive type 1 (SWS1) opsin and is sensitive to ultraviolet or near ultraviolet (UV) wavelengths.

Conflicting physiological and molecular evidence has confounded a mechanistic understanding of tetrachromacy in hummingbirds. Here we sought to address this issue using the Broad-tailed Hummingbird (*Selasphorus platycercus*; BTH), which has demonstrated color discrimination capabilities between ultraviolet and red, yellow, or green and has been long-studied in other contexts, including in ecological and physiological research (16). However, full length *sws1* sequences from genome assemblies have not been identified in hummingbirds, which conflicts with evidence from three hummingbird species of partial PCR-amplified sequence fragments *sws1* that encode 11 amino acids (4, 17).

The diurnal hummingbirds and swifts are phylogenetically nested within the primarily crepuscular superorder Strisores, which includes nightjars, frogmouths, and oilbirds (18). The order Caprimulgiformes, within the Strisores, consists of nocturnal or crepuscular birds sister to the hummingbirds and swifts. The European Nightjar (*Caprimulgus europaeus*), of the Caprimulgiformes, seems to have retained a full-length, functional copy of *sws1* in genome assemblies (5). One possibility for the exclusion of *sws1* from hummingbird and swift genomes is the difficulty in assembling genes with repeat-rich introns and/or high GC content that are notorious in birds (19).

Two SWS1 proteins have been characterized across birds. In one type, $\lambda_{\max}$ of SWS1 is in the violet range (VS-type) at 388–455 nm and the other type is in the ultraviolet range (UVS-type) at 355–380 nm (20, 21). Both types are most consistently distinguished by a single amino acid substitution at position 90, which is sufficient to shift the $\lambda_{\max}$ into the UV range (22). Models using electroretinogram data suggest a better fit with a UVS cone type in hummingbirds. The partial PCR fragments of *sws1* suggest that at least three hummingbird species encode the VS SWS1 type (14, 21, 23). Because the species examined by the electroretinogram and PCR-amplification data did not overlap, it is possible that there is variation among hummingbird species in the presence/absence of VS and UVS-type opsins. Nevertheless, electroretinogram data in the green-backed firecrown (*Sephanoides sephaniodes*) yielded $\lambda_{\max}$ values of 370, 440, 508 and 560 nm, implying the presence of four single cone cell types (23).

Oil Droplets

The arrays of often brightly colored oil droplets in each cone cell type are a prominent feature of avian retinas (24). The light that reaches and excites each cone cell's characteristic opsin is transmitted as a function of the types and concentrations of these carotenoids in the cone cell inner segment (8). Middle wavelength or green sensitive (M-cone) cone cells typically contain the pigment zeaxanthin or lutein, an ingested form of carotenoid that is the primary component of yellow oil droplets (Y-type). Red-sensitive L-cone cells, in contrast, have a diagnostic red oil droplet (R-type) containing astaxanthin or cis-astaxanthin, derived from zeaxanthin by ketolases such as CYP2J19 (25). Carotenoid composition is more variable in the short-wavelength sensitive S-cone cells and depends on the particular opsin protein present. Blue-sensitive S-cone cells expressing SWS2 usually have colorless oil droplets (C-type) containing the pigment galloxanthin. This pigment is synthesized from zeaxanthin via the enzymes BCO2 and RDH12 (6). S-cone cells expressing SWS1 are transparent due to lack of oil droplet pigments (T-type) and typically absorb light in the ultraviolet-sensitive (UVS) or near ultraviolet (VS). The predominant carotenoid of each cone cell type (zeaxanthin, astaxanthin, or

galloxanthin) typically defines the limit of long-wavelength absorption whereas spectral tuning of shorter-wavelengths is achieved by additional complex mixtures of carotenoids (8).

Shifts between SWS1 opsin types are often followed by complementary shifts in peak sensitivities of SWS2-containing cells, increasing color discrimination capability between short

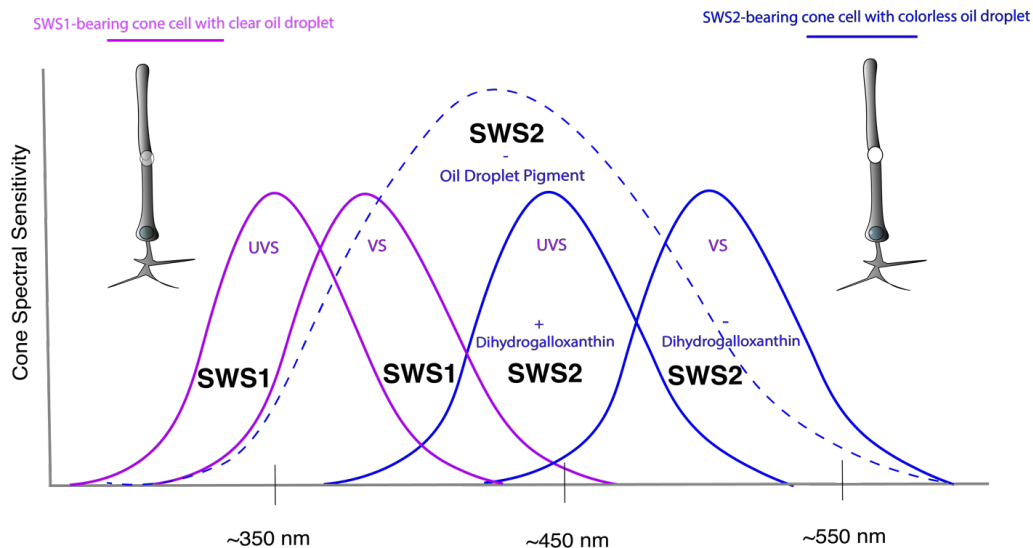


Figure 1. Diagram of the typical peak absorbances of SWS1 and SWS2 in birds based on oil droplet pigment composition. S-cone cells containing SWS1 typically have a transparent oil droplet (T-type) while those with SWS2 are colorless (C-type) and contain a mixture of galloxanthin and other pigments. Species with a UV-shifted SWS1 co-occur with short-wavelength shifted SWS2 as a result of increased dihydrogalloxanthin content in C-type oil droplets (6). SWS2-bearing cone cells that theoretically lack galloxanthin have a wider absorbance spectra sensitive to both blue and ultraviolet wavelengths.

and middle wavelengths (Figure)(6). This is accomplished by altering the pigment composition of oil droplets in SWS2-containing cells, or through a handful of spectral tuning sites in the SWS2 opsin protein (6, 7). Oil droplet pigment composition in S-type cells is modified by a third protein, RETSAT, which converts galloxanthin into 11',12'-dihydrogalloxanthin, which has a UV shifted absorbance spectra. When acting in combination, BCO2, RDH12, and RETSAT are sufficient to produce dihydrogalloxanthin, facilitating changes in the ratio of 11',12'-dihydrogalloxanthin to galloxanthin (6, 7). This ratio in SWS2-containing cells tends to be higher in UVS species compared to VS species. Because the amount of dihydrogalloxanthin present in the retina also correlates with transcript abundance, the presence of *retsat* in transcriptomic data may shed light on UV sensitivity in S-cone cells (6, 7).

The hummingbird eye is distinctive among birds, with a genomic architecture of color vision that likely involves complex regulation of opsins and pigment biosynthesis genes. In this study, we report a high quality genome assembly of the BTH in addition to sex-specific ocular transcriptomic assemblies to understand the structure and expression of key vision genes. The pattern of losses and gains of vision genes in the BTH led us to propose a possible mechanism for tetrachromacy through the regulation of carotenoid biosynthesis gene expression. These genomic resources, along with those already available, are aimed at providing researchers with tools to integrate foundational studies on hummingbirds and explore novel questions related to avian color vision. The BTH may help address long-standing questions of how color sensitivity

and discrimination are achieved in hummingbirds despite conflicting evidence for the presence and functionality of *sws1* in the genome (16, 26).

Methods

Genome Assembly

Comparative genomic analyses of color vision genes in birds report that gene losses and protein coding changes are variable both between and within avian families (4, 27). This suggests that even a closely related species like the Anna's Hummingbird (*Calypte anna*; AH; 2.44 mya) may not be an appropriate genomic comparison in the context of color discrimination behavior in the BTH (28). We therefore generated a high quality chromosome-level reference assembly for the BTH to avoid reference bias. Tissue for a BTH reference genome came from an adult male BTH that died after it collided with a window in Gothic, Colorado. This individual was immediately dissected, muscle tissue from primarily around the breast was removed and placed in a freezer-safe 2mL screw cap vial. A subset of tissue was flash frozen and sent to Dovetail Genomics to generate Hi-C and Chicago libraries. Remaining tissues were stored in 70% ethanol in 2mL screwcap vials stored in a -80° C freezer before overnight delivery on dry ice. DNA was extracted from heart tissue stored in 70% ethanol using a phenol-chloroform (PCI) clean using LN pulverization, Qiagen G2 Buffer, and PoteinaseK. The sample was incubated at 65°C for the PCI extraction followed by ethanol precipitation. PacBio RSII SMRT libraries were generated using the standard Pacbio CLR prep kit (Template prep kit v1). After size selection in the Blue Pippin, the average library size was 50 Kb fragments. Reads were on average 10 Kb and polymerase N50 was at ~21 kb with N50 of reads ~18 kb. We generated 68 Gb of data across 15 cells averaging 6 Gb per cell.

The initial assembly was generated with Canu v.1.7 (29) assuming a genome size of 1.1 Gb, given the recent AH assembly size (30). PacBio reads were mapped to the Canu output assembly with BLASR (commit 4323a52)(31), and the assembly was polished with PBDAGCon (commit 1a2f1e7)(31) two times, using the map4cns pipeline (commit dd89f52; <https://bitbucket.org/rokhsar-lab/map4cns>). Putative archaeal, bacterial, viral, and vector contamination was identified and removed by querying the assembly with BLAST+ (v2.6.0) against the respective RefSeq and UniVec databases, using general_decon.sh (v1.0), as previously described (32), (33). Mitochondrial sequences were also identified and removed by querying the assembly with BLAST+ (v2.6.0)(33) against the closest available mitochondrial assembly (NCBI NC_025786.1)(34), using mt_decon.sh (v1.0)(32). The decontamination removed seven contigs totaling 33.1 kb from the polished Canu assembly. PacBio reads were then mapped to the decontaminated assembly with BLASR (commit 4323a52)(30), using the map4cns pipeline (commit dd89f52; <https://bitbucket.org/rokhsar-lab/map4cns>), and the assembly was error corrected with a custom script LoReM (v1.0; <https://github.com/abmudd/Assembly>), which flagged six contigs with potential misassemblies based on low spanning coverage and high number of read terminals (starts and ends). These contigs were broken with BEDTools (v2.27.0)(35).

The assembly was organized into chromosomes with Chicago and Hi-C data using the Dovetail Genomics HiRise pipeline (36). The chromosome assembly was broken back into contigs with SeqKit (v0.7.2-dev) (37) due to the presence of haplotypic duplication caused by sequence differences in heterozygous regions, and was then filtered with the purge_haplotigs

pipeline (commit 0b9afdf; https://bitbucket.org/mroachawri/purge_haplotigs). The purge_haplotigs pipeline removed 3,759 contigs and a total of 152.4 Mb based on low, mid, and high read depth cutoffs of 7, 33, and 150, respectively, including the optional clip step. Hi-C reads were aligned to the remaining contigs with Juicer (commit 94ec691) (38), and the assembly was organized back into chromosomes, then manually corrected in Juicebox (v1.9.0) (39).

Gaps in the filtered chromosome assembly were filled with PacBio data using LR_Gapcloser (commit 156381a) (40). PacBio reads were then mapped to the assembly with pbalign (v0.4.1; <https://github.com/PacificBiosciences/pbalign>), and the assembly was polished with Arrow (v2.3.3; <https://github.com/PacificBiosciences/GenomicConsensus>) three times. Scaffolds smaller than 500 bp were removed from the final assembly release with seqtk (v1.3-r106; <https://github.com/lh3/seqtk>), and chromosomes and scaffolds were numbered in order of size using SeqKit (v0.7.2-dev)(37).

The final assembly was soft-masked with RepeatMasker (41) using Aves class-specific repeats derived from the combined libraries of Dfam_Consensus (release 20181026)(42) and RepBase (release 20170127)(43). The assembly was annotated using Gene Model Mapper (v1.6)(44) and BLAST+ (v2.9.0)(33) with the following assemblies and annotations as input evidence: AH (NCBI Annotation Release 100 of GCF_000699085.1)(30), Chicken (*Gallus gallus*) (NCBI Annotation Release 104 of GCF_000002315.6)(45), and Zebra Finch (NCBI Annotation Release 103 of GCF_000151805.1)(46). Coding nucleotide and peptide sequences were extracted using gff3ToGenePred and genePredToProt from the UCSC Genomics Institute (binaries downloaded March 5, 2019)(47) with postGeMoMa.py (v1.0)(32), and functional annotation was run with InterProScan (v5.34-73.0)(48). The Z chromosome was identified using a blastn (v2.6.0) search on our genome using the Chicken (NC_052572.1) Z chromosome as a query, identifying chromosome 4 in our assembly (33). We then assessed resequencing coverage on chromosome 4 for both birds used in transcriptome assembly using Qualimap to confirm sex (See Population Sampling and Transcriptome Assembly)(v2.2.2)(49).

Assembly metrics for the genome were done in comparison with the AH genome (30) using Quast v.5.0.2 (50). Assembly completeness was assessed on both genomes using BUSCO v.4.1.4 and the aves_odb10 database (51). Coverage was calculated using BMap (38.93) for all genomic libraries including PacBio, Chicago, and HiC data using the reformat function (52). We also implemented an alternative metric of assembly completeness with gene predictions in OrthoVenn2 using the Chicken, Turkey (*Meleagris gallipavo*), Mallard (*Anas platyrhynchos*), Zebra Finch, and Collared Flycatcher (*Ficedula albicollis*) for comparison (53).

Transcriptome Assembly

Genome assemblies may exclude intervals with high intron repeat content, which are notorious among avian genomes (19). Such genes have been recovered in some cases through transcriptome sequencing, which typically excludes introns. We assembled five high quality transcriptomes from the BTH to search for evidence for candidate vision-related genes from the relevant tissue. Tissue samples were obtained from four separate cryovials (2mL) and filled with RNALater (2mL) stored at -80°C: two eyes from an adult female, two eyes from a subadult male, one brain from an adult female, and one brain from a subadult male. Falcon tubes (50mL) filled with 100% ethanol and stored in the -80° C freezer contained breast muscle tissue from both individuals. Breast tissue was extracted using a Qiagen DNeasy Blood and Tissue Kit and sequenced on the NovaSeq 6000 using Kapa Hyperprep indexes. RNA was extracted from

tissues using the Rneasy Plus Universal Midi Kit with a total output of 64.67 micrograms. Libraries were then prepped with the Roche KAPA hyperprep kit or Takara smart seq v4 kit (low input) and sequenced on the NovaSeq 6000.

Read quality was assessed using Fastqc (v0.11.5) to assess base quality and adapter contamination (54). We then ran rcorrector to remove erroneous kmers produced by sequencing error (<https://github.com/mourisl/rcorrector>)(55). We discarded read pairs in circumstances where one read is low quality or complexity using the FilterUncorrectablePEfastq.py script from (<https://github.com/harvardinformatics/TranscriptomeAssemblyTools.git>). To avoid excessive adapter trimming via classic Illumina demultiplexing methods we used TrimGalore (v0.6.0) (56). We then removed rRNA reads by downloading the SILVA database using SSUParc and LSUParc sequences and were altered to have T's instead of U's (57). An index of this database was built prior to aligning sequence data with bowtie2 (58). Using unaligned reads, we reran fastqc to confirm there was no adapter contamination or overrepresented sequence and removed residual overrepresented sequences with the RemoveFastqcOverrepSequenceReads.py script from (<https://github.com/harvardinformatics/TranscriptomeAssemblyTools.git>). Each separate transcriptome was then assembled using Trinity v.2.8.5 (59). Assembly metrics were assessed using the TrinityStats.pl script in Trinity on the combined transcriptome assembly from both individuals (60). We quantified transcriptome reads by building a reference index and mapping reads using bowtie2 and converting to bam format with samtools (v1.6) to assess alignment metrics (58, 61, 62). We first removed adapter sequences using fastp (v0.23.2) and mapped trimmed reads to our genome with bwa (v0.7.17)(63, 64). PCR duplicates were then removed with Picard Tools (v.2.23.3) and sorted and indexed using samtools (v1.6)(62, 65).

Candidate Gene Search

We first implement high-throughput searches to identify candidate gene sequences followed by finer-grained analyses in cases where sequence is not identified. This first step included orthology searches that are effective at identifying partial sequences of incompletely assembled genes. Searches that did not identify gene sequences at this step were then followed up with less conservative alignment-based or read-based analyses that do not rely on assembly quality. A panel of genome assemblies for these analyses were chosen to contain members of the Strisores in addition to the Zebra Finch as an outgroup to assess shared vision gene content (Supplementary Table 1).

First-pass high throughput searches were focused solely on opsin genes with smRecSearch (<https://github.com/docmanny/smRecSearch.git>)(66). We first generated an annotation file using five known visual opsin genes along with their coordinates from the Zebra Finch genome (bTaeGut1.4.pri)(Supplementary Table 2). This bed file was then converted into a fasta file using bedtools getfasta (v.2.28.0) to simplify gene names of interest, which were then combined with the remaining masked Zebra Finch annotations using bedtools maskfasta (35). This method runs a BLAT search of each genome and transcriptome using a protein query and a translated nucleotide database of each genome for the forward search. We used protein fasta sequences of vision opsin genes from the Zebra Finch (Supplementary Table 3) as a query and a genome or transcriptome as the input database. Following each search, a reverse BLAT search used the aligned subject from the previous step and a nucleotide-nucleotide alignment between the subject and the Zebra Finch genome. The program then reports whether the aligned region is most similar to the annotated gene of interest relative to other genes, with the requirements that

reciprocal best hits need to have at least a score of 0.1, 80% identity, and a query span of 10% of the gene length.

Additional searches were conducted using BLAST v.2.7.1+ with less conservative default parameters using a larger panel of vision-related genes in addition to the vision opsins. A tblastn search was run on each genome and transcriptome using a protein fasta for each gene of interest (Supplementary Table 4). The exception to this was a blastn search of the *cyp2j19* gene cluster (KX024636) which can contain tandem paralogs in some species. Both BLAST searches were conducted in each genome and transcriptome database. Genes in the *cyp2j2-like* cluster were first identified in the genome using a tblastn search using CYP2J19 protein sequence as a query. All positive alignments that had a positive reverse blastp alignment to *cyp2j19*, *cyp2j2-like*, or *cyp2j2* and were located upstream of *hook1* were then identified as members of the *cyp2j2-like* cluster. Finally, annotated gene sequences for each member of this cluster in the BTH genome were then aligned to each ocular transcriptome with a blastn search to identify isoforms of each gene.

Alternative read-based methods were implemented for cases that did not identify genic sequence with alignment-based methods. Gene searches of Illumina reads were then performed in these cases using exemplars from each family in the Strisores. Raw paired-end or single-end Illumina data were first downloaded from NCBI by providing a list of accession numbers (Supplementary Table 5) to the sratoolkit (v.2.11.0)(67). All available sequences for species represented in each family with the exception of hummingbirds and swifts were obtained in this way. We utilized paired-end Illumina data from five BTH individuals in our dataset and subsampled paired-end read data for swifts due to computational constraints (See *Population Sampling*). All reads were trimmed using fastp (v0.23.2) to remove Illumina adapters and were combined into a single fastq file for each family. Files were then converted to fasta format using seqtk (v.1.3) prior to a tblastn search with blast-legacy (v.2.2.26) using the read fasta as a database (33, 63).

We were unable to identify orthologous sequence for opsins with BLAT- or BLAST-based searches for a subset of genomes. The inability to detect sequence using alignment-based search methods can be the result of high heterozygosity, sequence length, or low read quality of Illumina reads. Trimmed reads from each family were also aligned to a closely related genome containing functional *sws1* to account for sequence that may not have been assembled. A consensus sequence was then determined using aligned reads filtered for mapping quality (MapQ 60). Then, a blastn search was performed on this consensus sequence in the NCBI database. *Sws1* was first identified in the European Nightjar genome assembly (bCapEur3.1) using a tblastn search (17). Illumina data for Caprimulgidae were first aligned to chromosome 5 of the European Nightjar (OU015528.1:80727133-82497438) as a control using Minimap2 (v.2.24)(68). Genome sequence data from the remaining families were aligned to the European Nightjar genome assembly using NextGenMap (v.0.0.5) due to high divergence between the European Nightjar and members outside of the Caprimulgidae (69). Alignments were filtered to visualize the region of interest using samtools and IGV, and a consensus sequence was taken for alignments with a mapQ of 60 overlapping the region containing *sws1* (OU015528.1:80727133-82497438:780414-784505)(62, 70). Coverage of *sws1* using paired-end reads from each family in the Strisores was visualized in R using the ggplot2 package (71, 72).

Genome Structure Analysis

The shared synteny among bird genomes was leveraged to determine whether genome structure is conserved in candidate genic regions and whether shared structural rearrangements have occurred in the evolutionary history of a group (73). Genomes containing genes absent in the BTH genome assembly were used as a reference to provide evidence for shared structural rearrangements in the Strisores. We were interested in large-scale structural changes, so only chromosome-level assemblies were included in analysis: Great Potoo (*Nyctibius grandis*), Chimney Swift (*Chaetura pelagica*), AH, and BTH (Supplementary Table 1). The European Nightjar was chosen as a reference because it is a member of the Caprimulgidae, which is sister to the rest of the Strisores and has a functional copy of *sws1* on Chromosome 5. All alignments to the European Nightjar genome assembly were performed using Minimap2 with the long assembly to reference mapping preset and a divergence threshold of 20%, as the Caprimulgidae diverged earliest from the rest of the Strisores (v.2.24)(68). The Chimney Swift genome assembly was only aligned to European Nightjar Chromosome 5 (OU015528.1) due to the high amount of fragmentation in the Swift genome with low N50 to improve visualization. All alignments were then exported in pairwise alignment format, then visualized using the R program dotPlotly (<https://github.com/tpoorten/dotPlotly.git>).

We detected a candidate deletion overlapping sequence containing *sws1* in the Nightjar reference, but were unable to resolve whether gaps in the genome-genome alignment was the result of a true deletion or misassembly. Putative deletions in query genomes overlapping the sequence containing *sws1* in the European Nightjar assembly were characterized by aligning resequencing data from either the AH or BTH to this region. 10X linked-read data for the AH data were obtained from Genome Ark and trimmed of adapters using fastp, then aligned to the region in European Nightjar genome assembly containing *sws1* (OU015528.1:80727133-82497438) using NextGenMap (63, 74). We viewed alignments in linked-read view in the candidate region (780414-784505) with IGV (70). We additionally performed the same filtering and viewing pipeline to the paired-end reads from five BTH individuals from another study listed in Supplementary Table 5. We then linked supplementary alignments and sorted by mapping quality. We did not apply mapping quality filters to either of these alignments as the divergence is high in this region.

Transcriptomic Analysis

We were unable to find *sws1* in the genome of the BTH using candidate gene search methods in line with previous studies (4). The assembly of ocular transcriptomes allowed us to search for *sws1* along with other vision genes in the event sequences were not assembled or fragmented. A major component of RNASeq data is mature RNA, which does not contain introns, and is not as susceptible to the exclusion of data due to the repeat-rich intronic sequences common in avian assemblies (19). Opsins are additionally often excluded from assemblies due to the reduction in read coverage from high GC content in Illumina data (4). However, these genes should be detectable given that LWS has the highest median GC content of the opsins in birds and is often recovered in genome assemblies. The same candidate gene search methods of ocular transcriptomic assemblies was performed on each candidate gene in the BTH genome assembly. The identity of the initial gene ID with the highest score from the alignment using alignment or read-based methods was confirmed by running a reverse BLAST search on the subject of the alignment in the NCBI blastp portal for each expressed isoform that was identified in the forward tblastn search. We measured the proportion of the total gene length as a metric of relative

completeness and to quantify the extent of partial sequence detection for all candidate genes using the full-length Zebra Finch protein homolog as the reference for maximum length.

We were also interested in characterizing relative gene expression to assess how color discrimination may occur in the context of missing vision genes. The abundance of each isoform was quantified using Salmon (v.1.8.0) by first generating a transcriptome index for each individual using a kmer size of 31 (<https://github.com/COMBINE-lab/salmon.git>). Reads passing filtering steps used for transcriptome assembly in Trinity were used as input for Salmon along with each ocular transcriptome.

Population Sampling

Paired-end read data are particularly helpful for detecting structural variants as the orientation of read pairs around candidate genes may not reflect the orientation of sequence in the reference genome of another species. Deletions generate a specific pattern in paired-end read alignments where pairs of reads align only to the flanks of a sequence when a gene is present in the reference and missing in the query. In order to take advantage of the presence of *sws1* in the European Nightjar genome assembly, we aligned high coverage paired-end read data from 5 BTH individuals to this region (See Genome Structure Analysis). Each sample was then extracted for DNA using a modified phenol-chloroform extraction protocol developed by Gwin Wogan. Extracted DNA was assessed for quality using the Broad Range Qubit, Gel Electrophoresis, and bioanalyzer. All sample libraries were generated with the Nextera DNA Flex kit and were then sequenced on the NovaSeq 6000 to an average whole genome coverage of 10-13x across samples.

Results

Genome and Transcriptome Assembly of a Male Broad-tailed Hummingbird

Coverage for this PacBio library was roughly 53x coverage, with Chicago and HiC data at 65x and 56x, respectively. The initial contig-level assembly output of Canu produced 5605 contigs with a summed length of ~1.1 Gb, within the normative range for bird genome sizes (Supplementary Table 6). Contig sizes of this initial assembly had an NG50 of ~1.5 Mb prior to scaffolding with HiC and Chicago data (Supplementary Table 7). The final chromosome-level assembly had the expected 33 chromosomes comparable to the high-quality AH genome (bCalAnn1_v1). The scaffolded assembly had a total of 484 scaffolds and an NG50 of 72 Mb, high contiguity and similar quality to the AH assembly (NG50 of ~74 Mb) (Supplementary Table 8). The largest contig in both assemblies was ~196-197 Mb. Following scaffolding and decontamination, genome size was modestly reduced to ~1.0 Gb.

Genome completeness was assessed using BUSCO evaluating the BTH genome assembly at ~91% completeness in comparison to 94% in the AH assembly (Supplementary Table 9). However, avian genome assemblies often have high missingness due to presence of high GC content and repeat-rich introns, so these values are not unexpected (19). Output from RepeatMasker identified retrelements and LINEs as the primary repeat type in this assembly (Supplementary Table 10). Many genomes show reduced completeness metrics in contrast to their corresponding gene sets which suggests limitations of BUSCO gene prediction and is often the case with highly derived taxa (75). We also assessed completeness with alternative methods based on gene orthology to evaluate the completeness of gene content. Protein annotations from gene predictions were input to OrthoVenn2 and suggested all but 43 clusters have orthology with

at least one other bird (53) (Supplementary Table 1). As for “missingness” (defined by clusters present in all other species but absent for the species in question), BTH annotations had lower missingness (607 clusters) than the turkey (779 clusters) or Zebra Finch (691 clusters) annotations. We show from this that the annotations and assembly for the BTH are sufficiently complete in genic regions.

The transcriptome assembly was based on a total of 126,386,833 reads, all of which were paired (Supplementary Table 11). Quality metrics were based on the assembled transcriptome from both pairs of brain and eye libraries. Of these paired reads, ~87% of all pairs aligned concordantly one or more times translating into a 97.48% total alignment rate. We identified a total of ~484,000 transcripts in the transcriptome assembly (Supplementary Table 11). Contig N50 was normal at ~4,000 base pairs (bp) and an average contig length of ~1,470 bp. Contig N50 was at ~1,480 bp and an average length of ~780 bp for the longest isoform of each gene in the transcriptome.

Candidate Gene Search

Search methods used across the Strisores revealed family-specific patterns of possible opsin gene loss with respect to the five visual opsins. A summary of candidate gene searches can be found in Table 1. The rhodopsin gene (*rh1*) was recovered in all genomes analyzed as well as in both ocular transcriptomes. In contrast, the presence/absence of *rh2* using alignment-based methods like BLAT or BLAST was more variable. No *rh2* sequences were identified in the Owlet-Nightjar (*Aegotheles bennettii*), Chuck-will’s-widow (*Antrostomus carolinensis*), European Nightjar, or Lesser Nighthawk (*Chordeiles acutipennis*) genome assemblies using smRecSearch due to its strict gene length parameters. Sequences of *rh2* were recovered in all families using BLAST. We detected *lws* in the genome assemblies of most species using smRecSearch or BLAST, but *lws* was only found in the reads of the AH (2019 assembly), Rufous Potoo (*Nyctibius bracteatus*), Band-tailed Nighthawk (*Nyctiprogne leucopyga*), and European Nightjar, indicating *lws* was not assembled in these genomes in congruence with the high GC content of *lws* (Supplementary Table 13). Sequences of *sws2* were recovered using one of these methods in all species except for members of the Caprimulgidae. Results for *sws1* were the most variable. Sequences of *sws1* were found using smRecSearch or BLAST in the genomes or reads of at least one member of each family with the exception of hummingbirds (Trochilidae), swifts (Apodidae), and owlet-nightjars (Agothelidae) (Table).

We then align-trimmed reads from these three families to the region containing *sws1* in the European Nightjar genome assembly. A blastn search of the NCBI database using the consensus sequence of this alignment found a reverse best BLAST hit to *sws1* for high quality mapping reads (MapQ 60) in the Chimney Swift and Owlet-Nightjar genome assemblies. Reads from both AH and BTH genome sequences in contrast, had reverse best blastn hits to *sws2* (Supplementary Table 14). We did not identify *sws1* transcripts in either ocular transcriptome of the BTH, but did identify transcripts for *rh1*, *rh2*, *lws*, and *sws2* (Table 1). Transcripts were additionally found for *rdh12*, *bco2*, *retsat*, *scarb1*, *cyp2j2*, and *cyp2j19*. Searches for *cyp2j19* in Strisores genomes identified sequences of the *cyp2j19* gene cluster in all families (Table 3).

Database (Genome)	Rh1	Rh2	LWS	Sws1	Sws2	Technology
Blue-throated Hillstar	Y	Y	Y	N	Y	Illumina
Anna's Hummingbird 2019	Y	Y	Y	N	Y	Pacbio,10x,Bionano,Arima
Anna's Hummingbird 2014	Y	Y	Y	N	Y	Illumina
Whiskered Treeswift	Y	Y	Y	Y	Y	Illumina
Chimney Swift	Y	Y	Y	Y	Y	Illumina
Tawny Frogmouth	Y	Y	Y	Y	Y	Illumina
Owlet Nightjar	Y	Y	Y	Y	Y	Illumina
Rufous Potoo	Y	Y	Y	Y	Y	Illumina
Great Potoo	Y	Y	Y	Y	Y	Pacbio,Illumina,Bionano,Arima
Oilbird	Y	Y	Y	Y	Y	Illumina
Band-tailed Nighthawk	Y	Y	Y	Y	N	Illumina
Chuck-will's Widow	Y	Y	Y	Y	N	Illumina
Lesser Nighthawk	Y	Y	Y	Y	N	Illumina
European Nightjar	Y	Y	Y	Y	N	Pacbio,10x,Arima2
Key		Search				
Brown		SmRecSearch				
Black		Not found				
Light Blue		BLAST				
Orange		Reads for Family				
Purple		Consensus from Pileup				

Table 1. Summary of opsin identification using candidate gene search methods in genome assemblies. All present sequences colored brown were detected using smRecSearch with a cutoff requiring 10% of the gene length to be present in the alignment. Positive hits using manual tblastn searches for genomes are colored blue, and those for tblastn searches of trimmed read data for the respective *family* are colored orange. Positive reverse best blastn searches of the NCBI database using consensus sequences are colored purple. Consensus sequences were determined from reads with high map quality (60) aligned to *sws1* in the European Nightjar genome.

Transcriptome	<i>sws1</i>	<i>sws2</i>	<i>lws</i>	<i>rh1</i>	<i>rh2</i>	<i>bco2</i>	<i>retsat</i>	<i>scarb1</i>	<i>rdh12</i>
Adult Female	NP	334/362	340/365	339/351	330/355	610/610	581/621	492/498	324/365
Subadult Male	NP	340/362	340/365	339/351	330/355	610/610	581/621	492/498	313/365

Table 2. Candidate vision gene presence/absence summary using manual tblastn searches in each BTH transcriptome. Proteins listed in Supplementary Table 4 were used as queries. Gene completeness was measured by indicating the proportion of aligned protein length over the maximum protein length using complete Zebra Finch protein lengths as a reference.

Family	Species	Assembly	Assembly-type	<i>sws1</i>	<i>cyp2j19</i> cluster
Trochilidae	<i>Calypte anna</i>	bCalAnn1_v1.p	Genome	NP	Present
Trochilidae	<i>Selasphorus platycercus</i>	BTHv1	Genome	NP	Present
Apodidae	<i>Chaetura pelagica</i>	ChaPel_1.0	Genome	NP	Present
Hemiprocneidae	<i>Hemiprocne comata</i>	ASM1339685v1	Genome	NP	Present
Aegothelidae	<i>Aegotheles bennettii</i>	ASM1339812v1	Genome	NP	Present
Podargidae	<i>Podargus Strigoides</i>	ASM1339975v1	Genome	NP	Present
Nyctibiidae	<i>Nyctibius grandis</i>	bNycGra1.pri	Genome	NP	Present
Steatornithidae	<i>Steatornis caripensis</i>	ASM1339822v1	Genome	Present	Present
Caprimulgidae	<i>Caprimulgus europaeus</i>	bCapEur3.1	Genome	Present	Present

Table 3. Summary of *sws1* search results using a manual tblastn search in representative genomes of each family in Strisores. Also depicted are results for *cyp2j19* gene cluster identification from a blastn search with nucleotide sequence of the *cyp2j19* gene cluster from Zebra Finch as a query. Genes that did not have reverse blastp alignments are denoted as NP (Not Present).

Genome Structure Analysis

We aligned all chromosome-level genomes in the Strisores to the European Nightjar genome assembly to identify large-scale structural rearrangements specific to hummingbird genomes. This alignment revealed that hummingbird genomes have experienced large-scale chromosomal rearrangements relative to chromosome 5 in the European nightjar genome, which is the location of *sws1* in the European Nightjar genome. This particular rearrangement was

specific to the AH and BTH genomes and we found collinear alignments of the other chromosomes (Supplementary Figure 2). Visualizing these genome-genome alignments indicated high sequence homology with the Zebra Finch, which did not have clear large-scale rearrangements relative to the European Nightjar, except on Chromosome 1. Similarly, alignment of the Great Potoo genome showed a lack of large-scale rearrangements. Both the AH and BTH had large structural changes to sequence homologous with chromosome 5 of the European Nightjar genome, corresponding to sequences present on scaffold 72 in the BTH assembly. The Chimney Swift genome assembly, with a lower N50, was instead aligned only to the European Nightjar chromosome 5 to increase resolution of scaffold rearrangements. The Chimney Swift showed no large-scale rearrangements within this chromosome (Supplementary Figure 3).

We used paired-end reads from available Illumina sequences for the BTH and linked reads from the AH to identify localized family-specific structural changes to *sws1* and its surrounding sequence. A tblastn search with a Zebra Finch SWS1 sequence query confirmed the boundaries of *sws1* on chromosome 5 in the European Nightjar reference genome assembly. Two patterns emerged after aligning paired-end Illumina data for 5 individuals of the BTH to this region. Coverage was low to absent from the combined read data within the gene boundaries of European Nightjar *sws1*. In contrast, the flanks of *sws1* in the reference European Nightjar had considerable coverage (Figure 2). The coloring of linked-read alignments in IGV suggests the presence of many smaller-scale inversions and translocations/duplications on the flanking regions of the sequence. The reads with the highest mapping quality were characteristic of a deletion where read pairs span the flanks of the reference sequence, and do not overlap the gaps between supplementary alignments (alignments that don't map in a linear fashion) of this region. Linked reads from AH were also useful for analyzing structural data because these reads are effectively longer and therefore more likely to span the structural feature. These data echo the results for the BTH, indicating a deletion of *sws1* and many structural rearrangements relative to the European Nightjar reference sequence.

We have shown that AH and BTH genome assemblies likely contain a deletion in the region containing *sws1*, but we were also interested in determining whether this structural change was specific to hummingbirds. We performed the same paired-end read alignments using reads from exemplar species of other families besides hummingbirds in Strisores. When we aligned trimmed Illumina data to the same region of the European Nightjar genome assembly, we detected a pattern contrary to both hummingbird species. Coverage across this region was uniform in regions overlapping *sws1* coding sequence for all families, in contrast to Trochilidae which only had coverage on the flanks of *sws1*. We also saw an expected slight dip in coverage compared to the flanking sequence in all families due to the higher GC content. Far fewer data were present for Steatornithidae and Hemiprocridae, which is reflected in the genome-wide low coverage for these groups, albeit with sequence identification and coverage of *sws1*. Across all families within the Strisores using available paired-end read data, reads map to this region without evidence of a deletion, suggesting this pattern may be specific to hummingbirds (Figure 3).

Annotation of the *cyp2j2-like* gene cluster helped us determine which genes in this region are paralogous to *cyp2j19* for downstream transcriptomic analysis. A blastn search of the BTH genome using a *cyp2j19* gene cluster query located a predicted gene on chromosome 9 that had a reverse best BLAST hit to *cyp2j19* in the NCBI database. We identified two predicted genes downstream of *cyp2j19* from the BTH annotation file. The annotated BTH coding sequences

from *cyp2j19* and both downstream genes were used to perform a blastn search in the NCBI database, with positive hits to *cyp2j19*, *cyp2j2*, or *cyp2j2-like* in the NCBI database. All predicted genes between *cyp2j19* and *hook1* were designated as the *cyp2j2-like* gene cluster (excluding *hook1*) as in Zebra Finches, where *cyp2j40* lies downstream of two *cyp2j19* paralogs.

Transcriptomic Analysis

Analysis of both ocular transcriptomes revealed that a subset of key vision genes had low to no expression. A broad tblastn search of all vision-related genes in the ocular transcriptomes detected expression of *rh1*, *rh2*, *sws2*, *rdh12*, *bco2*, *scarb1*, and *retsat* (Supplementary Table 15). We did not detect expression of *sws1* and little *cyp2j19* expression. Sequence homologous to the *cyp2j19* gene cluster was present in the genome, but the majority of highly expressed isoforms mapped back to one of the *cyp2j2-like* genes following a tblastn search (Table 4). In both transcriptomes, we identified isoforms of each candidate gene and their normalized expression quantity (Supplementary Table 15). We found three isoforms of *bco2* in both transcriptomes and each had a normalized abundance of 24-27 transcripts per million (TPM). There were 4-5 *rdh12* isoforms in the two assemblies; females had a single highly expressed isoform (129 TPM), and males had two (36, 95 TPM). A single highly expressed isoform of *retsat* was present in the female eye and male eye at ~10-17 TPM. Two highly expressed isoforms of *scarb1* were present in both the male and female eye (14-27 TPM).

A high number of *cyp2j2-like* isoforms were present in both assemblies, in the female having 11 isoforms, and 5 isoforms in the male using CYP2J19 protein sequence in a tblastn search. Only one isoform was identified in each assembly from a tblastn search that had a reverse protein blast to *cyp2j19*, both of which had low expression (0.5-0.8 TPM). Blastn searches of

Gene	RBB	Location
14054	<i>cyp2j2-like</i>	Chr9:3259056-3262728
14055	<i>cyp2j2</i>	Chr9:3264872-3268792
14056	<i>cyp2j19</i>	Chr9:3247811-3256027

Table 4. Gene ID for annotated genes in the *cyp2j2-like* gene cluster. The ID of each gene was determined using a blastn search of the NCBI database with the predicted CDS for each gene.

each transcriptome using the gene sequence of each annotated *cyp2j2-like* gene in the BTH assembly. Only a single isoform of *cyp2j2-like* genes from this family was highly expressed in either assembly (13-24 TPM). One isoform had a reverse BLAST hit to *cyp2j19* in each assembly but had very low expression at 0.5-0.8 transcripts per million (Supplementary Table 15.). The most highly expressed *cyp2j2-like* isoform in both assemblies had a reverse protein BLAST hit to *cyp2j2-like* with other variably expressed isoforms with BLAST hits to *cyp2j2* or *cyp2j2-like*. Annotated gene sequence for the three *cyp2j2-like* genes (14054-14056) were used as queries in a blastn search of each transcriptome. Only two isoforms in females and three in males matched gene 14056, identified as *cyp2j19* in the BTH genome and ranged up to only 1.9 TPM (Supplementary Table 16). Highly expressed isoforms instead mapped to genes 14054 and 14055, which were identified in previous steps as *cyp2j2* and *cyp2j2-like*.

Expression of the vision opsins were consistent between the two ocular transcriptomes. Both *rh1* and *lws* had high expression in both assemblies with only one isoform each (*rh1*:109-136 TPM, *lws*:28-62 TPM). The lowest expression of the opsins was found in *rh2* (≤ 7 TPM). In both assemblies, *rh2* had four isoforms, two of which were truncated. The *sws2* gene was present in two isoforms in females (9,41 TPM), and three in males (13-16 TPM). Three isoforms with similar expression were found in males, one of which had an effective length half the other two.

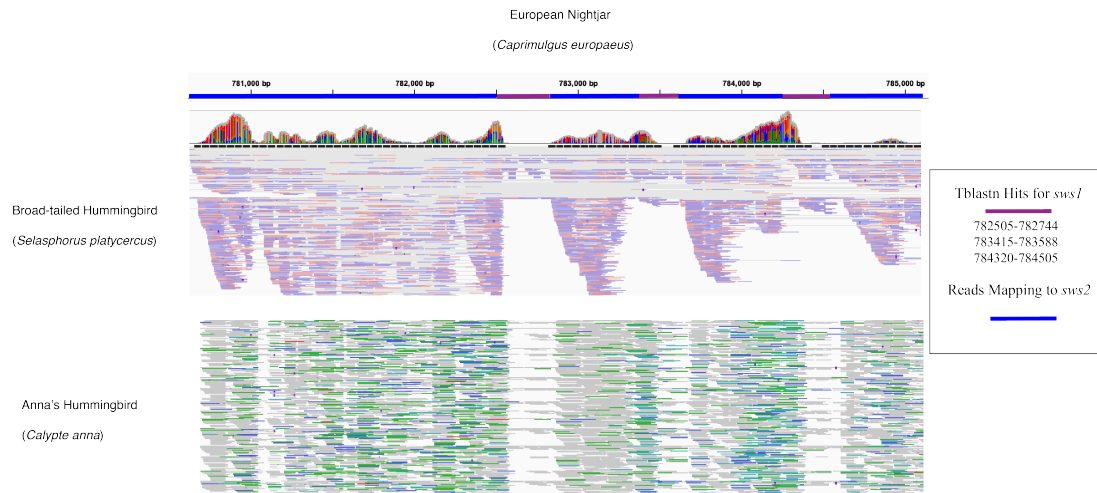


Figure 2. Evidence for deletion of *sws1* in the BTH and AH using reads aligned to the region containing *sws1* in the European Nightjar genome. Reads were aligned to this region and sorted by mapping quality using paired-end Illumina data from 5 BTH individuals (Top). 10X linked reads from the AH were aligned to the same region (bottom). The top panel of each plot shows linked supplementary reads with the bottom panel showing primary reads. Segments labelled with a blue line refer to sequence identified in tblastn searches of the BTH and AH genomes. All reads aligning to surrounding sequence are considered artifactual as a consensus of these reads maps back to *sws2*.

Discussion

The newly assembled genome and sex-specific ocular transcriptome assemblies of the BTH suggest a complex regulation of pigment and opsin composition of cone cells. We provide evidence for a genomic deletion of the UV opsin gene (*sws1*) and propose an alternative mechanism for tetrachromatic color vision in light of these data and ongoing behavioral research in the BTH.

Manifold examples of adaptations to the various photic environments birds inhabit have been documented, demonstrating that optimization of color discrimination has been achieved through changes to a shared genetic toolkit. These changes include coding changes that spectrally tune color sensitivity or deletions of genes that increase spectral sensitivity or reduce functional costs (4, 5, 7). Expression of genes can also be modified through cis-regulatory changes, subfunctionalization of gene clusters (76), or spatiotemporal variation within the retina based on a species' ecology or changes in ambient light (77, 78).

Losses or reduced functionality of visual genes can likely be attributed to the costs associated with their expression. For example, pigmentation of oil droplets may aid in color discrimination by filtering light reaching the opsins, but this also reduces the incident number of photons reaching them, decreasing sensitivity. Opsins too are associated with costs to the neural circuitry in particular cone cells which may be metabolically expensive to maintain, or may contain residue changes advantageous in a

common ancestor that make reversal mutations difficult (79). Even the composition of ocular media creates constraints with respect to ultraviolet light. A higher transmittance of light may permit more ultraviolet light to reach cone cells, but it also increases photo-oxidative damage to the retina and chromatic aberration at UV photoreceptors (80).

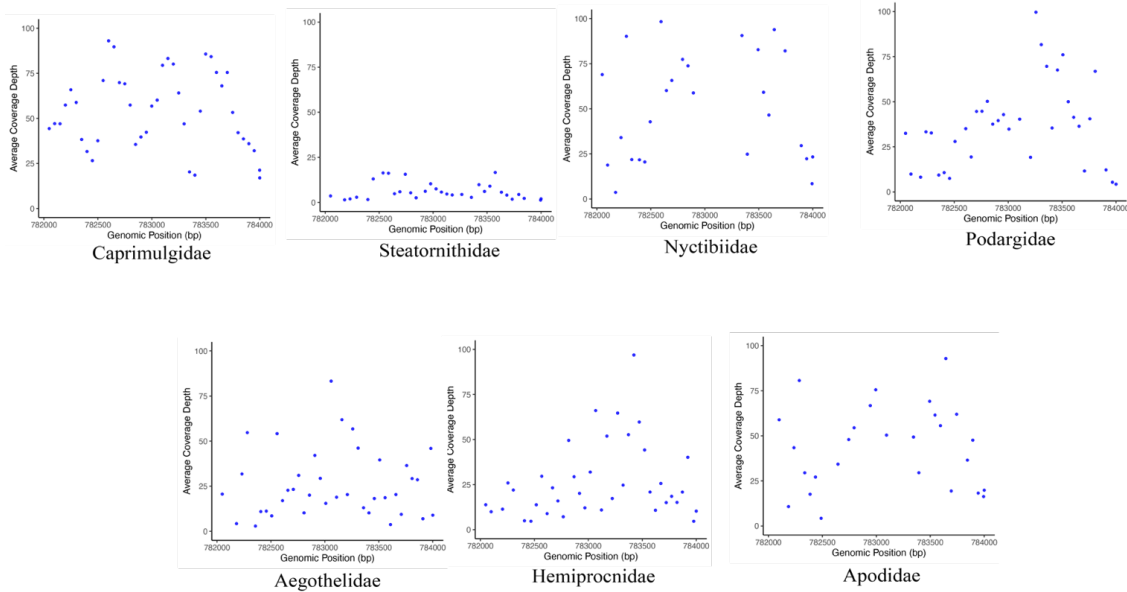


Figure 3. Coverage of reads plotted in 50 base pair windows across the length of Nightjar *sws1*. Publicly available Illumina sequences were compiled for each family in Strisores and mapped to Chromosome 5 of the European Nightjar genome assembly, which contains the *sws1* gene. Illumina data were few for Oilbirds, resulting in overall lower coverage. The consensus sequence in all cases aligned to *sws1*, in contrast to hummingbirds.

Ultraviolet Vision

Birds as a group have evolved the ability to see ultraviolet light, but this is not a property of all birds, with the majority of nonpasserine birds having violet-sensitive photoreceptors and ocular media that filters out UV light (81). One such group of nonpasserines, the hummingbirds and swifts, are thought to have violet-sensitive *sws1*-pigments, yet they can differ greatly in their ocular transmittance of UV light, which ultimately limits the amount of UV available to photopigments. However, ocular transmittance in the UV is particularly variable between species and likely a function of their specific photic environments (82–84).

The diurnal hummingbirds and swifts are nested within a primarily nocturnal or crepuscular group of birds, the Strisores (85). Mammals and the Strisores are ancestrally nocturnal or crepuscular, but only in mammals is nocturnality associated with the loss of *sws1* (79). Many nocturnal birds still retain a functional *sws1* gene, including the nightjars (Caprimulgidae) (5). This suggests that nocturnality is not sufficient to account for the loss of UV sensitivity and is likely associated with costs correlated with nocturnality.

The most parsimonious phylogenetic scenario suggests that nocturnality evolved in the stem-lineage of this group, with a reversal to diurnality in hummingbirds and swifts. Such reversals could involve dynamic copy number loss and gain associated with a shift to diurnality and the attendant reacquisition of color vision (86). However, it is possible the relaxed constraint

on *sws1* function may have occurred through changes in ocular transmittance in addition to shifts to nocturnality in an ancestor of hummingbirds. For example, owls transmit far more UV light in ocular media than diurnal raptors (83). Likewise, the ocular media of Green-backed firecrests, a forest-dwelling hummingbird, is UV-transparent, unlike that of swifts, which forage in open environments, the latter showing evidence of retaining *sws1* in this study. However, due to the variable light environments inhabited by hummingbirds, this is not expected to be the case for all hummingbirds and may be reflective of a common ancestor. The loss of *sws1* has also been recorded in diurnal birds including the cinereous vulture (*Aegypius monachus*), black-winged kite (*Elanus caeruleus*), little owl (*Athene noctua*), yellowhead (*Mohoua ochrocephala*), and brown creeper (*M. novaeseelandiae*), which suggests that loss of this opsin may be more widespread than previously understood (87, 88).

Hummingbirds are sensitive to UV light, which was discovered in 1972 when a single violet-eared hummingbird (*Colibri serrirostris*) was found to detect 382 nm, but not 363 nm light (12). Three additional species of hummingbirds also distinguished 370 nm UV light from darkness (89). Hummingbirds perceive and discriminate a wide range of color mixtures such as purple and other mixtures with UV, suggesting interactions between nonadjacent cone types contribute to color vision, although it still remains unclear whether hummingbirds can distinguish blue and ultraviolet light (16, 90). Simulations of measured electroretinogram data conducted in the green-backed firecrest and ruby-throated hummingbird predict the presence of four single cone types, including a predicted UV-sensitive cone with 5% participation (23). These electroretinogram data predict four single cone types in hummingbirds, but this will need to be confirmed using modern microspectrophotometric methods.

Although the BTH perceives UV light, our evidence suggests a deletion of *sws1* shared by both the BTH and AH. The use of the European Nightjar as a reference genome assembly, which has *sws1*, allowed us to leverage sequence similarity between the BTH, AH and this species along the flanks of *sws1* sequence and the fact that synteny is widely conserved among birds to infer a deletion (Figure 2) (73). Genome-genome alignments of the Zebra Finch and four members of Strisores to the European Nightjar genome showed a large chromosomal rearrangement of sequence homologous to Chromosome 5 specific to the hummingbirds (Supplementary Figure 2). We aligned available reads in NCBI to the Nightjar *sws1* from each family in Strisores and found coverage of this region, with a consensus sequence most similar to *sws1* of the opsins, in all groups besides hummingbirds (Figure 3; Supplementary Table 14). This finding comported with evidence that GC content in *sws1* is high, likely explaining why it is not present in many assemblies and why coverage was slightly lower within the genic region than the flanks in these groups (4). The opsins *rh1* and *rh2* are typically present in avian genome assemblies, whereas *lws*, *sws1*, and *sws2* are more often recovered as partial sequences. The fragmentation or misassembly of these three genes may be the result of their significantly higher GC content (4) or that they are located in GC rich regions such as microchromosomes. We find GC content between 60-68% in *lws* and *sws2* in the BTH assembly, both present on Scaffold 242, potentially explaining why these two genes have not been present in hummingbird genomes to date.

We were unable to recover *sws1* sequences in trimmed Illumina reads from BTH, which may contain sequence not incorporated into genome assemblies. Search methods used across the Strisores revealed family-specific patterns of possible opsin gene loss with respect to the five visual opsins. A summary of candidate gene searches can be found in Table 1. The rhodopsin gene (*rh1*) was recovered in all genomes analyzed as well as in both ocular transcriptomes. In

contrast, the presence/absence of *rh2* using alignment-based methods like BLAT or BLAST was more variable. No *rh2* sequences were identified in the Owlet-Nightjar (*Aegotheles bennettii*), Chuck-will's-widow (*Antristomus carolinensis*), European Nightjar, or Lesser Nighthawk (*Chordeiles acutipennis*) genome assemblies using smRecSearch due to its strict gene length parameters. Sequences of *rh2* were recovered in all families using BLAST. We detected *lws* in the genome assemblies of most species using smRecSearch or BLAST, but *lws* was only found in the reads of the AH (2019 assembly), Rufous Potoo (*Nyctibius bracteatus*), Band-tailed Nighthawk (*Nyctiprogne leucopyga*), and European Nightjar (*Caprimulgus europaeus*). Perhaps the most compelling evidence that *sws1* is absent in hummingbirds is the lack of *sws1* transcripts in two ocular transcriptomes. All these data are in agreement with previous comparative genomic studies unable to find partial sequences of *sws1* in hummingbirds (4). It is possible that the length and variability of *sws1* sequences recovered by (21) can be explained by nonfunctional *sws1* sequences present in a GC-rich region. In fact, the absence of *sws1* in the Swift (*Chaetura pelagica*) genome is potentially due to GC richness, as reads from Apodidae aligned well to this region in the Nightjar genome, with a consensus sequence having a positive reverse BLAST to *sws1* (Supplementary Table 14).

Color vision in birds has been classified as either violet-sensitive (VS) or ultraviolet-sensitive (UVS) and can be accurately determined based on two amino acid sites in the SWS1 protein, sites 86 and 90 (81). PCR amplification and Sanger sequencing of this gene across birds indicated that at least 14 shifts from a VS to UVS visual system have occurred based on these specific sites. The unique ability of this protein to extend into the ultraviolet spectrum results from deprotonation of the Schiff base linkage at the retinal chromophore (22). The spectral tuning of SWS2, in contrast, is more variable. From a sequence level, site-directed mutagenesis experiments on SWS2 indicated that shifts in the wavelength of maximum absorbance can be the additive effect of up to 10 amino acid replacements (7). Shifts in the spectral tuning of SWS1 are often accompanied by a different mode of spectral tuning in SWS2 cones, mediated by the ratio of two apocarotenoids present in their oil droplets, galloxanthin and 11',12'-dihydrogalloxanthin (6).

Oil Droplets

If hummingbirds lack SWS1 protein, they would need to alter pigment compositions in SWS2 bearing cone cells, if SWS2 is responsible for UV perception. Transparent oil droplets typically harbor SWS1 devoid of carotenoids while colorless oil droplets in SWS2-bearing cells typically contain pigments acting as long-wavelength cutoff filters. The blue-throated hummingbird (*Lampornis clemenciae*) retina harbors C-type oil droplets present on the dorsal hemisphere of their retina (91). These colorless oil droplets typically contain galloxanthin, which are characteristic of SWS2-bearing cone cells. Galloxanthin is one of the most abundant carotenoids in the eye, is found in all pigmented oil droplets, and is likely an important short-wavelength filter in most cone cells (8). Colored oil droplets contain a predominant carotenoid characteristic of its droplet type, but oil droplet pigments contain mixtures of carotenoids that spectrally tune individual cells, particularly at shorter wavelengths (8). We are just beginning to understand how oil droplet variation contributes to avian color vision.

At odds with behavioral data suggesting hummingbirds can perceive ultraviolet light, the deletion of *sws1* implies an alternative mechanism of ultraviolet vision in birds. We propose a model with two scenarios. One possibility is that *sws2*-bearing cells have reduced pigmentation, broadening the sensitivity of SWS2 to include both blue and UV light (Figure 4). In this case,

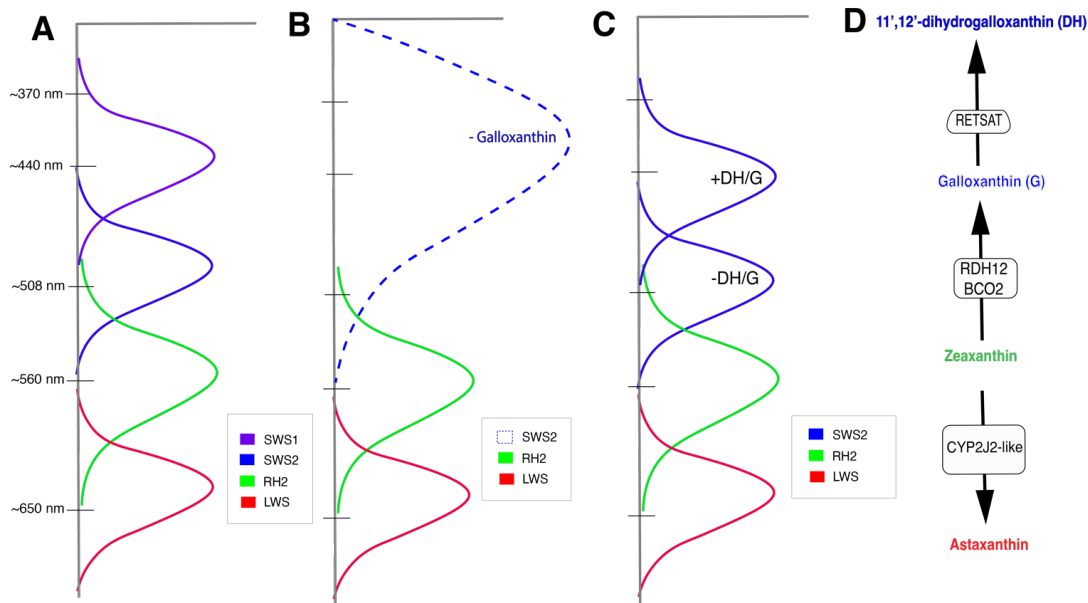


Figure 4. Theoretical alternative model for color vision in the BTH. A. Typical organization of cone cells in birds, with peak wavelength of absorption for opsins as a function of pigment composition. B. Alternative model of ultraviolet vision where S-type cone cells are unpigmented with a broad sensitivity to both UV and short wavelengths, lacking discriminability between the two. C. Second alternative model where a subset of *sws2*-bearing cone cells have a modified ratio of dihydrogalloxanthin to galloxanthin to shift absorption between S-type cells and optimize discriminability. D. Oil droplet pigments are derived from ingested forms of carotenoids such as zeaxanthin or lutein. The production of BCO2 and RDH12 are sufficient to generate galloxanthin where RETSAT further modifies galloxanthin into DH. CYP2J19 typically produces astaxanthin, but has low expression in the BTH. The regulation of astaxanthin concentration may occur through other genes in the *cyp2j2-like* gene cluster.

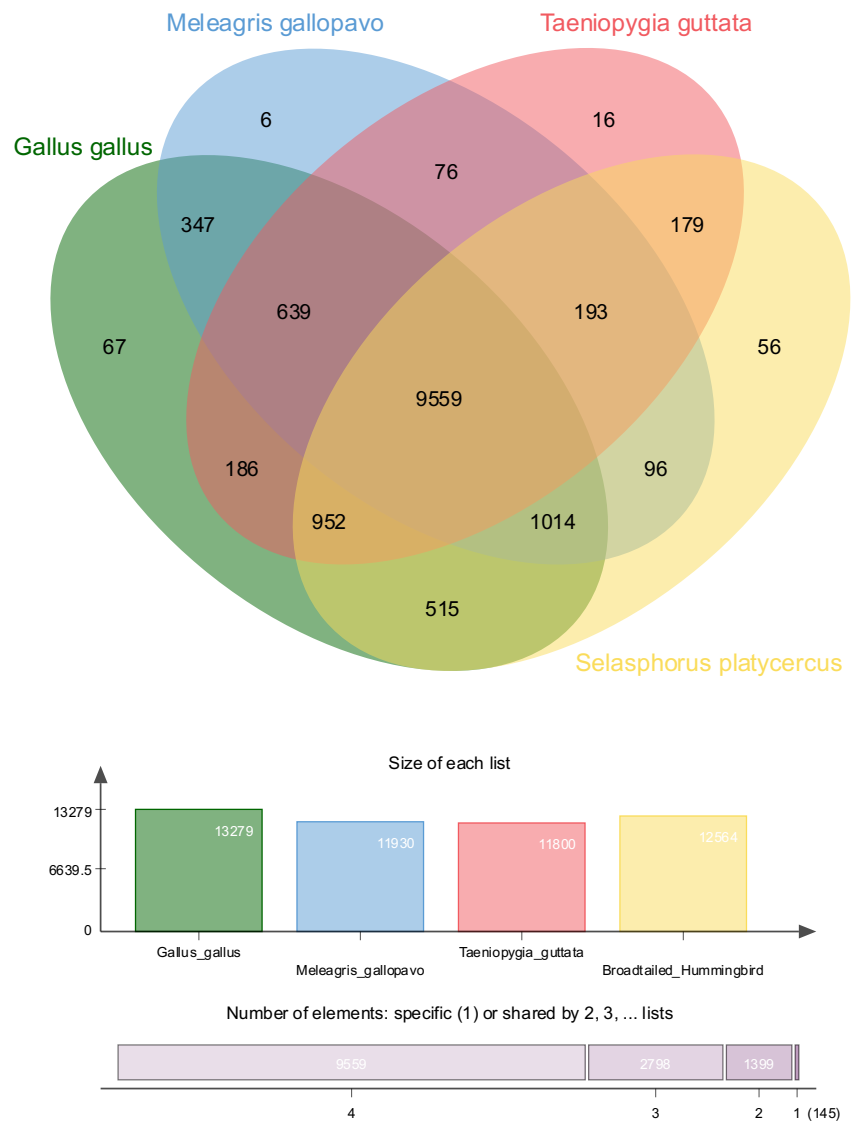
hummingbirds would theoretically have a reduced ability to discriminate blue and UV, which remains to be tested with behavioral experiments. Specifically, choice experiments utilizing variable short-wavelength light regimes. An alternative scenario would be that only a subset of *sws2*-bearing cells have reduced pigmentation, or differ in ratios of dihydrogalloxanthin to galloxanthin. This would reduce overlap in absorbance spectra, as with SWS2-bearing cones in other birds with UV-shifted SWS1 (8). It remains to be fully understood how oil pigmentation, ocular media transmittance, and opsin spectral tuning all interact in ways to optimize color discrimination ability. Integrating these data with molecular, behavioral and physiological observations, it may be the case that variable models exist for mechanisms underlying tetrachromacy in birds. Analogous to umami-derived sweet taste perception in hummingbirds, these findings suggest ecological adaptation of vision can be complex such that visual systems have the ability to evolve compensatory abilities for previously lost vision genes (94).

Supplementary Methods

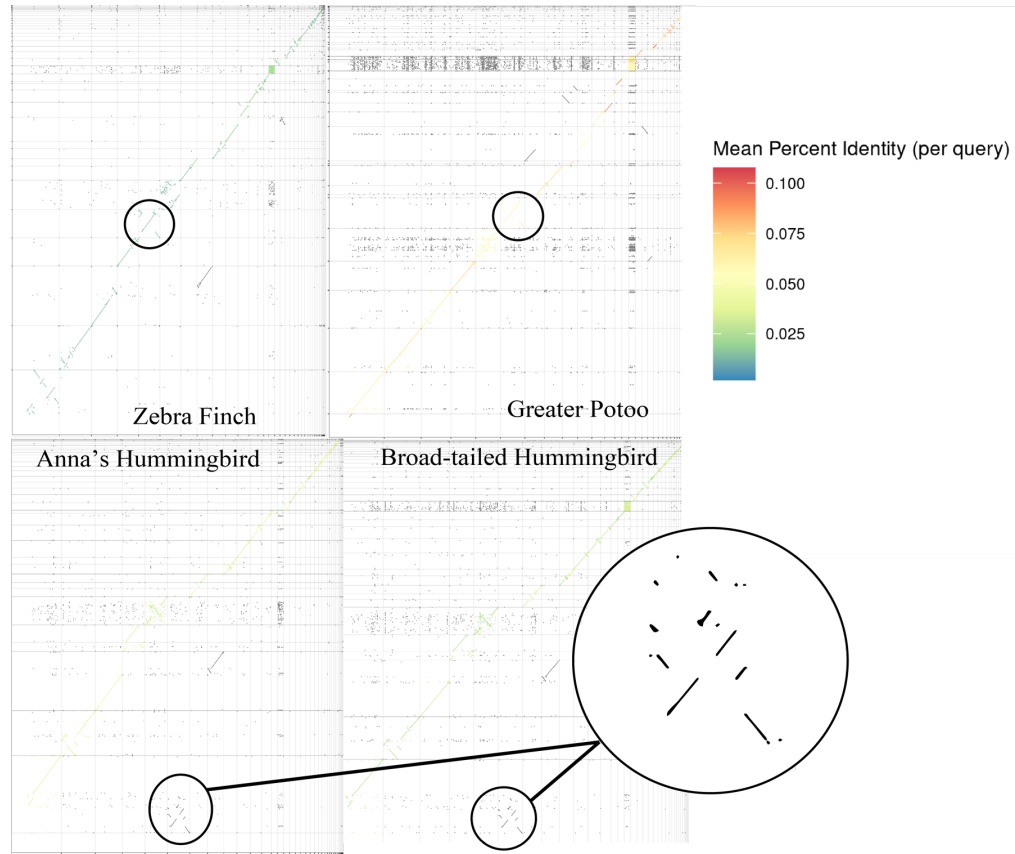
Population Sampling

All sampling of birds was done between 2015 and 2019 in Gunnison County, Colorado under federal and state permits and local and university animal care committee approvals. A feeder was set up at each sampling site, with 40% sucrose for 3-5 days prior to sampling to get local birds accustomed to the feeder. We set up a bonnet trap designed by Lee Rogers that forms a cylindrical net around the feeder when the trip line is released. A subset of these birds were banded, but for the unbanded subset, we only sampled from these sites in a single year and identified recaptures through toenail clips.

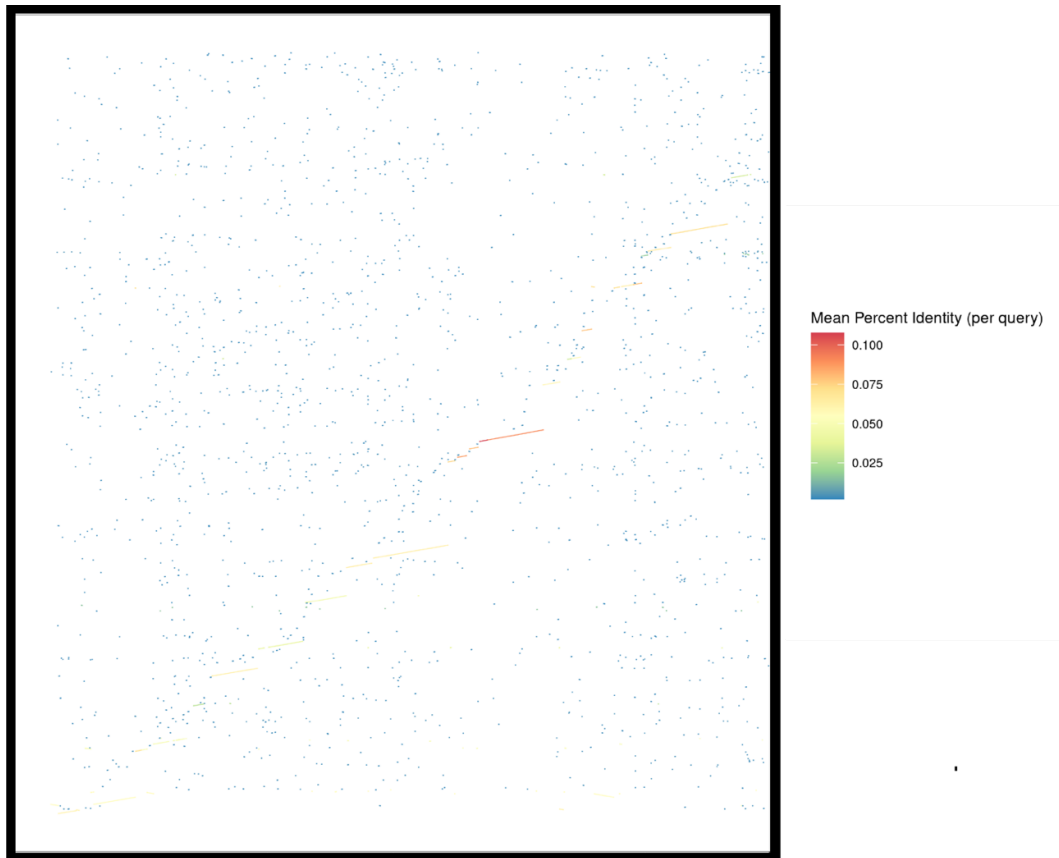
A single drop of blood (10 microliters) was collected from each bird for DNA analysis using the toenail clipping method (92). We clipped 0.5mm of one toenail with sterile fine scissors and collected 1% or less of body weight of blood in a 100 microliter heparinized capillary tube. The blood was then stored in a 2 mL Cryovial filled with Longmire's Buffer (93). Kwik Stop Styptic Powder is then applied with slight pressure to the foot. Scissors are sterilized between birds with 100% ethanol and dried. The bird was observed closely to assure that all bleeding has stopped before being released. Approximately 300 microliters of blood and buffer was pipetted from each individual cryovial and placed in a lysis buffer for 4-8 hours for DNA extractions.



Supplementary Figure 1. Output of Orthovenn2 depicting the shared number of orthogroups between the BTH, Turkey , Zebra Finch, and Chicken genomes. Number of elements (y-axis) shared were determined using predicted protein annotations as input. Note the improvement over the Turkey and Zebra Finch.



Supplementary Figure 2. Chromosome level genome assemblies of Zebra Finch, Great Potoo, BTH, and AH aligned to the European Nightjar genome (x-axis). The black circle denotes the location of chromosome 5 in the European Nightjar, which contains *sws1*. Notice in hummingbirds, this particular chromosome has largescale rearrangements. Percent identity is depicted as a color gradient with warmer colors denoting higher similarity.



Supplementary Figure 3. Chromosome level genome assembly of the Chimney Swift aligned to the European Nightjar chromosome 5 (x-axis). The entire square denotes the aligned portion of the Chimney swift genome to the European Nightjar chromosome 5. Notice there is no evidence of large-scale rearrangements, similar to the Potoo with high similarity to the Nightjar genome.

Supplementary Table 1. List of genomes used for analyses including the species name, assembly name, and assembly type. Note that some Strisores families have multiple representative species. Some analyses used only one representative species with the highest quality genome per family.

Common name	Species Name	Assembly	Assembly-type
Broad-tailed Hummingbird	<i>Selasphorus platycercus</i>	AfEyev1	Transcriptome
Broad-tailed Hummingbird	<i>Selasphorus platycercus</i>	ImEyev1	Transcriptome
Broad-tailed Hummingbird	<i>Selasphorus platycercus</i>	AfBrainv1	Transcriptome
Broad-tailed Hummingbird	<i>Selasphorus platycercus</i>	ImBrainv1	Transcriptome
Broad-tailed Hummingbird	<i>Selasphorus platycercus</i>	BTHv1	Genome
Anna's Hummingbird	<i>Calypte anna</i>	bCalAnn1_v1.p	Genome
Anna's Hummingbird	<i>Calypte anna</i>	ASM69908v1	Genome
Black-breasted Hillstar	<i>Oreotrochilus melanogaster</i>	ASM1340099v1	Genome
Chimney Swift	<i>Chaetura pelagica</i>	ChaPel_1.0	Genome
Whiskered Treeswift	<i>Hemiprocne comata</i>	ASM1339685v1	Genome
Barred Owlet-Nightjar	<i>Aegotheles bennettii</i>	ASM1339812v1	Genome
Tawny Frogmouth	<i>Podargus Strigoides</i>	ASM1339975v1	Genome
Great Potoo	<i>Nyctibius grandis</i>	bNycGra1.pri	Genome
Rufous Potoo	<i>Nyctibius bracteatus</i>	ASM1339659v1	Genome
Oilbird	<i>Steatornis caripensis</i>	ASM1339822v1	Genome
Band-tailed Nighthawk	<i>Nyctiprogne leucopyga</i>	ASM1339939v1	Genome
Chuck-will's-widow	<i>Antrostomus carolinensis</i>	ASM70074v1	Genome
Lesser Nighthawk	<i>Chordeiles acutipennis</i>	ASM1339963v1	Genome

European Nightjar	<i>Caprimulgus europaeus</i>	bCapEur3.1	Genome
Zebra Finch	<i>Taeniopygia guttata</i>	bTaeGut1.4.pri	Genome
Chicken	<i>Gallus gallus</i>	bGalGal1.mat.broiler.GRCg7b	Genome

Supplementary Table 2. Location of annotations used as a reference for smRecSearch. The subject of each successful alignment in was used in a reverse BLAT search using these annotations. A BLAT hit with a unique reverse BLAST identity lists one of the genes below.

Genome Annotation		
Zebra Finch (bTaeGut1.4.pri)		
Gene	Scaffold	Region
SWS1	NC_044212.2	173851-177337
SWS2	NC_054763.1	223025-229747
LWS	NC_054763.1	232429-239964
RH1	NC_044224.2	375526-388004
RH2	NC_044238.2	1934860-1938075

Supplementary Table 3. List of accessions for protein fastas used for the forward BLAT search in smRecSearch. All fasta sequences come from Zebra Finch.

Protein	Accession	Type
SWS1	AAF63463.1	Protein
SWS2	NP_001070165.1	Protein
LWS	NP_001070170.1	Protein
RH1	NP_001070163.1	Protein
RH2	NP_001070164.1	Protein

Supplementary Table 4. List of protein names and fasta file accession numbers for queries used in manual tblastn searches of genomes and transcriptomes. Genomes or transcriptomes were used as databases with the following proteins as queries in a tblastn or blastn search with default parameters.

Gene	Species	Accession	Length	Type
SWS1	<i>Taeniopygia guttata</i>	NP_001070172.1	346 aa	Protein
SWS2	<i>Taeniopygia guttata</i>	NP_001070165.1	362 aa	Protein
CYP2J19	<i>Quelea quelea</i>	AUG98151.1	498 aa	Protein
BCO2	<i>Gallus gallus</i>	XP_004948199.2	610 aa	Protein
LWS	<i>Taeniopygia guttata</i>	NP_001070170.1	365 aa	Protein
RH1	<i>Taeniopygia guttata</i>	AAF63461.1	351 aa	Protein
RH2	<i>Taeniopygia guttata</i>	NP_001070164.1	355 aa	Protein
RETSAT	<i>Taeniopygia guttata</i>	XP_030145976.3	621 aa	Protein
SCRB1	<i>Taeniopygia guttata</i>	XP_041575336.1	498 aa	Protein
RDH12	<i>Geospiza fortis</i>	XP_030920637.1	365 aa	Protein
CYP2J40	<i>Taeniopygia guttata</i>	XP_032605727.2	1002 aa	Protein
CYP2J19 gene cluster	<i>Taeniopygia guttata</i>	KX024636	26177 bp	Nucleotide

Supplementary Table 5. List of accession numbers for all Illumina data downloaded from NCBI. Each accession number represents either paired-end or single-end sequencing reads. All samples for Trochilidae were instead obtained from five high coverage BTH part of another study.

Accession	Family
SRX6757342	Podargidae
SRX6757341	Podargidae
SRX6757340	Podargidae
SRX6757339	Podargidae
SRX6695534	Podargidae
SRX6695457	Aegothelidae
SRX6695163	Aegothelidae
SRX6695092	Aegothelidae
SRX6694934	Aegothelidae
SRX322385	Apodidae
SRX322384	Apodidae
SRX322383	Apodidae
SRX322382	Apodidae
SRX322381	Apodidae
SRX322380	Apodidae
SRX322379	Apodidae
SRX322378	Apodidae
SRX322377	Apodidae
SRX322376	Apodidae
SRX322375	Apodidae
SRX6695032	Hemiprocnidae
SRX6695022	Hemiprocnidae
SRX6695021	Hemiprocnidae
SRX6733568	Nyctibiidae
SRX6733567	Nyctibiidae
SRX6694924	Nyctibiidae
SRX6695415	Nyctibiidae
SRX6695414	Nyctibiidae
SRX6695368	Nyctibiidae
SRX6608233	Steatornithidae
SRX6608107	Steatornithidae
SRX6608105	Steatornithidae
SRX6695864	Caprimulgidae
SRX6695365	Caprimulgidae
SRX6695115	Caprimulgidae

SRX6695114	Caprimulgidae
SRX336130	Caprimulgidae
SRX336129	Caprimulgidae
ERX5693574	Caprimulgidae
ERX5693571	Caprimulgidae
ERX5693570	Caprimulgidae
ERX5693572	Caprimulgidae
ERX5693573	Caprimulgidae
ERX5693569	Caprimulgidae
AL5_7_10_12	Trochilidae
AL3_7_3_10	Trochilidae
AL5_7_10_9	Trochilidae
AL2_7_7_10	Trochilidae
DR_7_6_10	Trochilidae

Supplementary Table 6. Assembly metrics of contig-level assembly output from Canu.

Canu Assembly Metrics	# Sequences	Total Length
Contigs	5605 Sequences	1172039395 bp
Unassembled	558685 Sequences	4020823072 bp
Bubbles	0 Sequences	0 bp
Repeats	291 Repeats	13537005 bp

Supplementary Table 7. Contig size distribution of Canu contig-level assembly.

Quartile	NG (bp)	LG (contigs)	sum (bp)
10	4858095	16	111450325
20	3700003	42	220537225
30	2589059	79	332386898
40	2046669	127	441017639
50	1570140	188	550299607
60	1155016	270	660147050
70	788330	384	770121784
80	433973	570	880214088
90	121140	1018	990055263
100	41849	2842	1100038182

Supplementary Table 8. Assembly metrics of the complete chromosome-level BTH genome assembly.

Assembly	Broad-tailed Hummingbird	Anna's Hummingbird (bCalAnn1_v1)
# Chromosomes	33	33
# contigs (≥ 0 bp)	484	159
# contigs (≥ 1000 bp)	483	159
# contigs (≥ 5000 bp)	406	158
# contigs (≥ 10000 bp)	354	158
# contigs (≥ 25000 bp)	180	134
# contigs (≥ 50000 bp)	82	83
Total length (≥ 0 bp)	1009456979	1059687259
Total length (≥ 1000 bp)	1009455980	1059687259
Total length (≥ 5000 bp)	1009342959	1059682482
Total length (≥ 10000 bp)	1008939709	1059682482
Total length (≥ 25000 bp)	1006016373	1059160479
Total length (≥ 50000 bp)	1002567468	1057264095
# contigs	484	159
Largest contig	196345723	197551010
Total length	1009456979	1059687259
GC (%)	41.52	41.49
N50	72349162	74081004
N75	23109348	23291945
L50	4	4
L75	11	12
# N's per 100 kbp	0.47	1519.01

Supplementary Table 9. BUSCO scores between the AH and BTH genomes using the Aves10 database.

BUSCO Type	<i>C. anna</i> %	<i>C. anna</i> n	<i>S. platycercus</i> %	<i>S. platycercus</i> n
Complete	94.70%	7897	90.80%	7567
Complete and Single-copy	94.40%	7872	90.50%	7544
Complete and Duplicated	0.30%	25	0.30%	23
Fragmented	1.20%	96	2.70%	226
Missing	4.10%	345	6.50%	545
Total BUSCOs Searched	8338	8338	8338	8338

Supplementary Table 10. Output of RepeatMasker summarizing the proportion of repeat element types in the BTH genome. The majority of repeats were identified as retroelements and LINES.

Element Type	Number of Elements	Length (bp)	Percent of Sequence
Retroelements	176864	67771673	6.71%
SINEs	5486	685185	0.07%
Penelope	100	20858	0.00%
LINES	156394	61020105	6.04%
CRE/SLACS	0	0	0.00%
L2/CR1/Rex	155587	60518382	6.00%
R1/LOA/Jockey	0	0	0.00%
R2/R4/NeSL	21	9000	0.00%
RTE/Bov-B	621	456629	0.05%
L1/CIN4	65	15236	0.00%
LTR elements	14984	6066383	0.60%
BEL/Pao	0	0	0.00%
Ty1/Copia	0	0	0.00%
Gypsy/DIRS1	0	0	0.00%
Retroviral	14813	6036932	0.60%
DNA transposons	14636	2264662	0.22%
hobo-Activator	2348	397666	0.04%
Tc1-IS630-Pogo	436	78210	0.01%
En-Spm	0	0	0.00%
MuDR-IS905	0	0	0.00%
PiggyBac	0	0	0.00%
Tourist/Harbinger	3816	357532	0.04%
Other (Mirage,P-element, Transib)	0	0	0.00%
Rolling-circles	0	0	0.00%
Unclassified	2352	414620	0.04%
Total interspersed repeats	NA	70450955	6.98%

Small RNA	1857	232436	0.02%
Satellites	1381	187565	0.02%
Simple repeats	271994	11890641	1.18%
Low complexity	60580	3364735	0.33%

Supplementary Table 11. Assembly quality metrics of the complete BTH transcriptome. This Transcriptome includes the eyes and brains of an immature male and adult female.

Complete Transcriptome Quality Metrics
126386833 reads
126386833 (100.00%) paired
→16976265 (13.43%) aligned concordantly 0 times
→17790782 (14.08%) aligned concordantly exactly 1 time
→91619786 (72.49%) aligned concordantly >1 times
16976265 pairs aligned concordantly 0 times
→1077185 (6.35%) aligned discordantly 1 time
→15899080 pairs aligned 0 times concordantly or discordantly;
31798160 mates make up the pairs
→6379690 (20.06%) aligned 0 times
→2945000 (9.26%) aligned exactly 1 time
→22473470 (70.68%) aligned >1 times
97.48% overall alignment rate

Supplementary Table 12. Assembly completeness metrics of complete BTH transcriptome.

Complete Transcriptome Assembly Metrics:
Total trinity 'genes': 337778
Total trinity transcripts: 483527
Percent GC: 44.86
Stats based on ALL transcript contigs:
Contig N10: 11872
Contig N20: 8725
Contig N30: 6782
Contig N40: 5362
Contig N50: 4143
Median contig length: 477
Average contig: 1474.05
Total assembled bases: 712740911
Stats based on ONLY LONGEST ISOFORM per 'GENE':
Contig N10: 9606
Contig N20: 6234
Contig N30: 4016
Contig N40: 2495
Contig N50: 1483
Median contig length: 356
Average contig: 778.94
Total assembled bases: 263108491

Supplementary Table 13. GC content measured in the most highly expressed isoform present in the immature male ocular transcriptome.

Gene	Isoform	GC Content
<i>lws</i>	TRINITY_DN21924_c0_g1_i1	60.10%
<i>rh1</i>	TRINITY_DN16814_c0_g1_i29	54.80%
<i>rh2</i>	TRINITY_DN16814_c0_g1_i25	53.80%
<i>sws2</i>	TRINITY_DN255_c1_g1_i4	68.60%

Supplementary Table 14. Illumina aligned to sws1 the European Nightjar genome were filtered for map quality, then a consensus sequence for reads aligned to the reference *sws1* coordinates was evaluated using a blastn search to the NCBI database. This table depicts the map quality filter and best reverse blastn hit for each consensus sequence taken from these alignments.

Genome	Map Quality	RBB - Gene
Anna's Hummingbird	60	<i>sws2</i>
Broad-tailed Hummingbird	60	<i>sws2</i>
Anna's Hummingbird	30	<i>pinopsin</i>
Broad-tailed Hummingbird	30	<i>pinopsin</i>
Anna's Hummingbird	0	<i>sws1</i>
Broad-tailed Hummingbird	0	<i>sws1</i>
Chimney Swift	60	<i>sws1</i>
Whiskered Treeswift	60	<i>sws1</i>
European Nightjar	60	<i>sws1</i>
Owlet Nightjar	60	<i>sws1</i>
Tawny Frogmouth	60	<i>sws1</i>
Great Potoo	60	<i>sws1</i>
Oilbird	60	<i>sws1</i>

Supplementary Table 15. List of isoforms from the immature male eye and adult female eye transcriptomes identified through a tblastn search using queries from Supplementary Table 4.

Transcriptome	Gene	Coverage	Length (nt)	Effective Length	TPM	NumReads
Adult Female Eye	bco2	TRINITY_DN1702_c0_g1_i16	2858	2564.103	24.915353	1848.91
Adult Female Eye	bco2	TRINITY_DN1702_c0_g1_i12	3424	3130.103	0.000000	0
Adult Female Eye	bco2	TRINITY_DN1702_c0_g1_i1	6316	6022.103	0.372710	64.958
Adult Female Eye	bco2	TRINITY_DN1702_c0_g1_i4	3362	3068.103	0.711126	63.144
Adult Female Eye	lws	TRINITY_DN7943_c1_g1_i2	1354	1060.103	62.153886	1906.908
Adult Female Eye	lws	TRINITY_DN7943_c1_g1_i1	1317	1023.103	2.828246	83.743
Adult Female Eye	rdh12	TRINITY_DN1617_c0_g2_i11	2788	2494.103	36.235797	2615.565
Adult Female Eye	rdh12	TRINITY_DN1617_c0_g2_i17	2696	2402.103	95.886326	6665.943
Adult Female Eye	rdh12	TRINITY_DN1617_c0_g2_i5	3495	3201.103	1.464623	135.687
Adult Female Eye	rdh12	TRINITY_DN1617_c0_g2_i6	2999	2705.103	5.494546	430.159
Adult Female Eye	retsat	TRINITY_DN5028_c0_g1_i1	2663	2369.103	17.767694	1218.227
Adult Female Eye	rh1	TRINITY_DN28_c0_g2_i4	3120	2826.103	1.854918	151.714
Adult Female Eye	rh1	TRINITY_DN28_c0_g2_i2	3341	3047.103	1.894895	167.104
Adult Female Eye	rh1	TRINITY_DN28_c0_g2_i1	2455	2161.103	136.114286	8513.192
Adult Female Eye	rh2	TRINITY_DN28_c0_g1_i2	2993	2699.103	0.228882	17.879
Adult Female Eye	rh2	TRINITY_DN28_c0_g1_i1	2897	2603.103	1.037593	78.168
Adult Female Eye	rh2	TRINITY_DN28_c0_g1_i4	1985	1691.103	1.203828	58.918
Adult Female Eye	rh2	TRINITY_DN28_c0_g1_i5	1851	1557.103	6.873015	309.726
Adult Female Eye	scrb1	TRINITY_DN25314_c0_g1_i6	2139	1845.103	14.103466	753.112
Adult Female Eye	scrb1	TRINITY_DN25314_c0_g1_i4	2019	1725.103	24.236605	1210.041
Adult Female Eye	scrb1	TRINITY_DN25314_c0_g1_i3	2414	2120.103	0.874885	53.681
Adult Female Eye	scrb1	TRINITY_DN25314_c0_g1_i1	4624	4330.103	1.531214	191.888
Adult Female Eye	sws2	TRINITY_DN1910_c0_g1_i3	1469	1175.103	41.518717	1411.995

Adult Female Eye	sws2	TRINITY_DN1910_c0_g1_i1	1351	1057.103	9.902813	302.963
Immature Male Eye	bco2	TRINITY_DN263_c0_g2_i11	2359	2051.451	27.592618	1538.91
Immature Male Eye	bco2	TRINITY_DN263_c0_g2_i6	1811	1525.451	0.000000	0
Immature Male Eye	bco2	TRINITY_DN263_c0_g2_i4	4468	4160.451	1.647989	186.403
Immature Male Eye	bco2	TRINITY_DN263_c0_g2_i8	5220	4912.451	2.597695	346.933
Immature Male Eye	lws	TRINITY_DN21924_c0_g1_i1	1400	1114.451	28.145224	852.756
Immature Male Eye	rdh12	TRINITY_DN2348_c0_g1_i14	5612	5326.451	0.000000	0
Immature Male Eye	rdh12	TRINITY_DN2348_c0_g1_i26	5527	5241.451	0.000000	0
Immature Male Eye	rdh12	TRINITY_DN2348_c0_g1_i8	4793	4507.451	0.000000	0
Immature Male Eye	rdh12	TRINITY_DN2348_c0_g1_i2	2605	2319.451	129.500650	8166.131
Immature Male Eye	rdh12	TRINITY_DN2348_c0_g1_i3	5589	5303.451	2.228777	321.354
Immature Male Eye	rdh12	TRINITY_DN2348_c0_g1_i13	4816	4530.451	2.339582	288.163
Immature Male Eye	rdh12	TRINITY_DN2348_c0_g1_i22	3401	3115.451	2.653117	224.717
Immature Male Eye	rdh12	TRINITY_DN2348_c0_g1_i24	3339	3053.451	3.297645	273.75
Immature Male Eye	retsat	TRINITY_DN5875_c1_g1_i3	2556	2270.451	10.949123	675.851
Immature Male Eye	retsat	TRINITY_DN5875_c1_g1_i1	992	706.458	0.000000	0
Immature Male Eye	retsat	TRINITY_DN5875_c1_g1_i4	751	466.319	0.000000	0
Immature Male Eye	retsat	TRINITY_DN5875_c1_g1_i5	696	412.054	0.722251	8.091
Immature Male Eye	retsat	TRINITY_DN5875_c1_g1_i2	2358	2072.451	3.094215	174.339
Immature Male Eye	rh1	TRINITY_DN16814_c0_g1_i29	2627	2341.451	109.984053	7001.224
Immature Male Eye	rh2	TRINITY_DN16814_c0_g1_i14	1729	1443.451	0.099560	3.907
Immature Male Eye	rh2	TRINITY_DN16814_c0_g1_i15	1711	1425.451	0.668016	25.888
Immature Male Eye	rh2	TRINITY_DN16814_c0_g1_i27	1744	1458.451	4.925816	195.312
Immature Male Eye	rh2	TRINITY_DN16814_c0_g1_i25	1726	1440.451	7.639033	299.155
Immature Male Eye	scrib1	TRINITY_DN1162_c0_g1_i1	2046	1760.451	21.274962	1018.244

Immature Male Eye	scrbl	TRINITY_DN1162_c0_gl_i3	2166	1880.451	27.615728	1411.815
Immature Male Eye	scrbl	TRINITY_DN1162_c0_gl_i2	1895	1609.451	0.462668	20.244
Immature Male Eye	scrbl	TRINITY_DN1162_c0_gl_i6	1775	1489.451	1.059963	42.922
Immature Male Eye	scrbl	TRINITY_DN1162_c0_gl_i5	4637	4351.451	1.876014	221.937
Immature Male Eye	sws2	TRINITY_DN255_c1_gl_i4	1549	1263.451	13.001604	446.596
Immature Male Eye	sws2	TRINITY_DN255_c1_gl_i13	1478	1192.451	14.137089	458.311
Immature Male Eye	sws2	TRINITY_DN255_c1_gl_i2	855	569.72	16.118287	249.655
Immature Male Eye	sws2	TRINITY_DN255_c1_gl_i16	1537	1251.451	0.000000	0
Immature Male Eye	sws2	TRINITY_DN255_c1_gl_i8	1567	1281.451	0.000000	0
Immature Male Eye	sws2	TRINITY_DN255_c1_gl_i7	1544	1258.451	1.149777	39.338

Supplementary Table 16. Summary table of isoforms in both ocular transcriptomes identified in a blastn search. All isoforms listed were identified using predicted genes from the BTH *cyp2j2-like* gene cluster as queries. Normalized transcript counts for predicted *cyp2j19* gene (14056) isoforms.

Transcriptome	Gene	Isoform	Length	E-value	TPM	NumReads
Female Eye	14056	TRINITY_DN6250_c1_g1_i3	len=3463	3.00E-105	1.980721	181.666
Female Eye	14056	TRINITY_DN6250_c1_g1_i2	len=717	3.00E-105	0.823722	10.129
Male Eye	14056	TRINITY_DN2972_c0_g1_i6	len=5121	1.00E-103	0.626222	82.324
Male Eye	14056	TRINITY_DN2972_c0_g1_i4	len=1191	1.00E-103	0.516331	12.71
Male Eye	14056	TRINITY_DN2972_c0_g1_i1	len=5120	1.00E-103	1.63296	214.626
Female Eye	14055	TRINITY_DN738_c0_g1_i9	len=7745	2.00E-86	1.831772	395.007
Female Eye	14055	TRINITY_DN738_c0_g1_i8	len=7747	2.00E-86	0	0
Female Eye	14055	TRINITY_DN738_c0_g1_i7	len=9910	2.00E-86	2.83648	789.391
Female Eye	14055	TRINITY_DN738_c0_g1_i6	len=8537	2.00E-86	5.379513	1283.355
Female Eye	14055	TRINITY_DN738_c0_g1_i27	len=9151	2.00E-86	0	0
Female Eye	14055	TRINITY_DN738_c0_g1_i21	len=9906	2.00E-86	0	0
Female Eye	14055	TRINITY_DN738_c0_g1_i20	len=9908	2.00E-86	9.231261	2568.521
Female Eye	14055	TRINITY_DN738_c0_g1_i18	len=6649	2.00E-86	13.189013	2425.762
Female Eye	14055	TRINITY_DN738_c0_g1_i13	len=6644	2.00E-86	0	0
Female Eye	14055	TRINITY_DN738_c0_g1_i12	len=3503	2.00E-86	4.502134	418.134

Female Eye	1405 5	TRINITY_DN738_c0_g1_i1 1	len=990 4	2.00E-86	3.115628	866.537
Female Eye	1405 5	TRINITY_DN738_c0_g1_i1	len=664 2	2.00E-86	3.649789	670.54
Male Eye	1405 5	TRINITY_DN2972_c0_g1_i 5	len=268 6	2.00E-86	24.48145 5	1597.678
Male Eye	1405 5	TRINITY_DN148624_c0_g1 _i1	len=592	7.00E-26	1.453358	12.306
Female Eye	1405 4	TRINITY_DN738_c0_g1_i9	len=774 5	6.00E-87	1.831772	395.007
Female Eye	1405 4	TRINITY_DN738_c0_g1_i8	len=774 7	6.00E-87	0	0
Female Eye	1405 4	TRINITY_DN738_c0_g1_i7	len=991 0	6.00E-87	2.83648	789.391
Female Eye	1405 4	TRINITY_DN738_c0_g1_i6	len=853 7	6.00E-87	5.379513	1283.355
Female Eye	1405 4	TRINITY_DN738_c0_g1_i2 7	len=915 1	6.00E-87	0	0
Female Eye	1405 4	TRINITY_DN738_c0_g1_i2 1	len=990 6	6.00E-87	0	0
Female Eye	1405 4	TRINITY_DN738_c0_g1_i2 0	len=990 8	6.00E-87	9.231261	2568.521
Female Eye	1405 4	TRINITY_DN738_c0_g1_i1 8	len=664 9	6.00E-87	13.18901 3	2425.762
Female Eye	1405 4	TRINITY_DN738_c0_g1_i1 3	len=664 4	6.00E-87	0	0
Female Eye	1405 4	TRINITY_DN738_c0_g1_i1 2	len=350 3	6.00E-87	4.502134	418.134
Female Eye	1405 4	TRINITY_DN738_c0_g1_i1 1	len=990 4	6.00E-87	3.115628	866.537
Female Eye	1405 4	TRINITY_DN738_c0_g1_i1	len=664 2	6.00E-87	3.649789	670.54
Male Eye	1405 4	TRINITY_DN2972_c0_g1_i 5	len=268 6	1.00E-88	24.48145 5	1597.678

Genomic Architecture of Foraging Traits in a Wild Hummingbird Population

Introduction

Optimal foraging theory predicts that plant-pollinator traits are co-adapted to maximize feeding efficiency of pollinators and pollen transfer of plants (1). However, generalized foraging strategies are the rule among pollinators rather than the exception (2). Despite the diffuse nature of most known pollination networks, variation in suites of pollinator traits, from tongue lengths in bumblebees to color vision capabilities in birds, are correlated with variation in suites of floral traits (3, 4). Within both pollinators and plants, assortments of traits often covary to reach new coadapted maximums (5), and as a result may be reflected in overlapping genomic architectures via correlated selection between traits (6). Variation for traits subtending floral pollination syndromes in Columbine flowers (*Aquilegia* spp.), such as petal color and spur size for example, show a shared genomic architecture, consistent with this hypothesis (7). However, no studies have examined the genomic architecture of correlated foraging traits in plant's animal pollinators, which often include variation in behavior and morphology.

Here we addressed this gap using the Broad-tailed hummingbird (BTH; *Selasphorus platycircus*), which has been the focus of detailed studies in eco-physiology, foraging ecology, pollination biology, and animal behavior studies since the early 1970's at the Rocky Mountain Biological Laboratory in Gothic, Colorado. The BTH seasonally migrates from Central Mexico to the Sierra Nevada Mountains, Madrean "Sky Island" Archipelago, and Rocky Mountains each summer and has a breeding season that coincides with the emergence of morphologically variable flower species that bloom in a predictable sequence (8). Furthermore, long-term flowering phenology studies have provided fine-scale resolution of the abundance and timing of floral availability within its range (9).

The BTH is a generalist nectar forager within the bee hummingbird clade. As a result, the flowers of many different plant species are used as nectar sources by the BTH. This results in a highly diffuse network of nectar sources, including ornithophilous, atypical flowers, and sap fluxes (10). One of the most well-studied flowers in the context of its foraging ecology at the RMBL is scarlet gilia (*Ipomopsis aggregata*), which has a long, tubular flower that has responded evolutionarily in a suite of floral traits to strong phenotypic selection from hummingbird pollination (11). Atypical flowers used as nectar plants like those of *Mertensia ciliata* and *Mertensia fusiformis* are much shorter, but are used in the early and late summer as food sources (12). Although hummingbirds visit a large range of flower lengths, scientists have noted the correspondence between bill length and average flower length, suggesting some functional role for bill morphological variation within species (13). Bills of hummingbirds are also used to capture insects, create nests, feed offspring, thermoregulate, and defend territories.

Bill morphology in birds, which is directly related to foraging performance, consequently influences habitat use, movement, and breeding behavior (14). Studies in passerines have revealed that shifts in bill morphology occur over short time scales following environmental change, and such shifts proceed using standing genetic variation (15, 16). Evolution of bill size is well-studied in birds, wherein different size morphs facilitate niche partitioning within species (17). Therefore, heritable components of bill morphological variation could be maintained in environments with highly variable food availability. Hummingbirds in particular use floral nectar

as their primary food source and are subject to spatially and temporally varying floral communities, especially in species with migratory populations like the BTH.

The size and shape of hummingbird bills vary widely among individuals of the same species, including dimensions of length, width, and curvature, each of which influences foraging efficiencies at different flowers (13, 18). In the Ruby-throated hummingbird (*Archilocus colubris*), another bee hummingbird and close relative of the BTH, longer-billed birds reached deeper into flowers than shorter-billed birds, but short-billed birds made fewer insertion errors than longer-billed birds. This implies that distinct optimal foraging strategies are expected within natural populations of hummingbirds that reflects heritable variation in bill traits (18).

Across the growing season in Gothic, Colorado, flora with large nectar rewards exhibiting the typical “hummingbird syndrome” emerge later in the summer from late June until August (19). This phenology could leave longer-billed birds at a foraging disadvantage in the early summer where atypical flowers are more common. The incredible spatial memory of hummingbirds (20) may then facilitate the highly variable seasonal latitudinal and longitudinal migrations of this species (21) when seeking out profitable nectar plants.

In turn, variation in migration phenologies are heritable in populations of many birds, resulting in life history polymorphisms wherein the available niche space varies by arrival time (22). For example, in European blackcaps (*Sylvia atricapella*), the differential exploitation of food resources seems to have been facilitated by co-divergent migration phenotypes and beak morphology, suggesting at least generational niche specialization (23). If bill morphology provides individual birds with distinct advantages at common flowers, local migration should covary with the phenology of flowers that optimize individual foraging efficiency.

In cases where variation in bill morphology and migration phenology are each heritable and subject to consistent correlated selection, genomic architectures of the two traits could become shared or even co-selected across the genome (24). Using a genome assembly from Chapter 2, I then captured and obtained DNA samples from 510 BTH individuals. All of these individuals were phenotyped for morphological traits and a subset for the presence of pollen on the bill from two important nectar plants. I then resequenced whole genomes from each of these 510 birds at 3x coverage, which allowed us to uncover the genomic architecture of three inter-related foraging traits in this species: bill morphology, migration phenology, and floral preferences.

We found that phenology of flowering influences phenology of arrival at the breeding site during migration, and that beak length predicts arrival date. We then found a link between bill length with the prevalence of pollen in hummingbird-specialized and atypical flowers that vary in their flowering phenologies.

Using quantitative a genomics approach, we found that variation in ecologically important phenotypes are highly heritable and quantitative in nature, with varying degrees of polygenic and oligogenic components, and include loci with pleiotropic effects on morphology and behavior. We also found that the genomic architectures of distinct sets of foraging traits in these animal pollinators are both partially overlapping and exhibit patterns consistent with being co-selected across the genome through long-range linkage disequilibrium (LD). Genetic correlations between traits can be the result of LD generated by co-selection (7), or pleiotropy (24), which can then facilitate adaptations as a suite or syndrome of traits, such as those important for increased foraging efficiency or pollen transfer from pollinators.

Our results reveal how behavioral and morphological phenotypes can co-evolve in natural populations in the context of an optimal foraging framework. We found that genomic modules underlying variation in both bill length and arrival time co-vary within a predictably seasonal environment. We speculate that these genetically correlated and co-selected suites of variable foraging traits may promote subtle foraging niche differences between individual, generalist hummingbirds. Specifically, we hypothesize that heritable variation in bill length feedbacks affects foraging efficiencies at nectar plants, which in turn influences the evolution of migratory arrival time.

Methods

Phenotypes

We quantified phenotypic variation across a panel of phenotypes to characterize the genomic architecture and estimate the heritable component of two sets of foraging traits in hummingbirds: prevalence of pollen and bill morphology. For the morphological traits each bird was measured with calipers and photographed from both a side and dorsal perspective for different phenotypes. A Canon EOS Rebel T7i was set up with a small tripod on a flat surface for both profile and dorsal images a camera with the lens about 1–3 meter away vertically from the individual. We set up a blank sheet of white paper as a backdrop to improve contrast along with a centimeter ruler for size calibration. A microscope slide box was placed underneath the bill to level the specimen and covered this in white paper to prevent glare. We set the individual on its side for profile images with the bill propped up on the stand and levelled to the box surface. The bird was held such that the bill was flat on the box surface for dorsal images. Full gape, exposed culmen, culmen width, culmen curvature, culmen depth, and mandible width were measured using ImageJ, calibrating each image with the photographed ruler.

The majority of bill measurements were conducted on the culmen, the location where feathers end on the maxilla. Bill depth, bill width, and exposed culmen were all measured in this way. Bill depth was measured as the height of the bill, including the mandible, at the culmen. Exposed culmen was measured from the culmen to the bill tip in profile view and bill width was measured at the culmen on the maxilla in dorsal view. We included another measure of bill length, full gape, as the length of the bill from the base of the gape to the bill tip. Full gape was measured in addition to exposed culmen because feather growth could vary among individuals on the maxilla and may limit the depth a bill can be inserted into a flower. We also measured the width of the gape at the mandible base for the same reason.

Bill curvature was measured from the profile view in ImageJ (25). We measured curvature by marking three equidistant points along the upper ridge of the maxilla, spanning from the end of the feathers at the culmen to the bill tip. A circle was then fit to these three points, allowing us to then obtain the radius of that circle. Curvature (C) was calculated by $C = 1/(\text{radius})$ using methods from (18). A second method of curvature, denoted as F-curvature, fit a circle using the same method to the ridge along the upper edge of the bill instead of the midline.

Some measurements included could not be completed in ImageJ so they were measured manually. Wing chord was the only phenotype measured exclusively using calipers, measured from the shoulder to the wingtip. We also counted the number of iridescent feathers on adult females as most did not have this trait, but varied in number among those that did.

Profile and dorsal images of each bird were also used to examine the shape of each bill independent of size. We first cropped raw images to include only the head of the bird using ImageJ (25). We then drew a white line perpendicular to the bill at the culmen and ran a custom macro to convert the outline of the bill into a black and white image. JPG images were first converted to BMP format and a chaincode was run to convert all BMP files into an NEF file (26). Normalized elliptical Fourier descriptors (EFDs) were loaded into R and subsequent analyses were performed using the Momocs v.1.3.0 R package (27). In each case, the same analysis was performed for both dorsal and profile images separately. Asymmetrical variation in shape was eliminated using the `rm_asy` function, which set all B and C harmonic coefficients to 0. We conducted principal components analysis (PCA) on the males and females jointly. The variation along the first five morphometric principal components (PCs) was visualized using `PCcontrib` function. Only the first two principal components of profile and dorsal shape were considered for GWAS because these PCs explained the majority of phenotypic variance for each trait.

We collected pollen samples from each individual bird to test for downstream associations between bill morphology and flower morphology. Pollen samples were obtained from most BTH individuals we live-captured by using a pair of tweezers to obtain a 1mm x 1mm piece of fuchsin gel from a pre-prepared petri dish. The 1mm x 1mm piece of gel was dabbed along the feathers of the crown, neck, breast, and upper and lower bill of each individual in the same manner and with the same effort across individuals. Each piece of pollen-coated fuchsin gel was placed in 1.5 ml centrifuge tubes and labeled with the date and sex of the individual. Each sample was then immediately placed on a glass slide and hovered over a 95% ethanol flame for ten seconds. Immediately afterwards, a glass coverslip was placed on top of the sample. We generated a reference library of local flora in Gunnison County and identified pollen from two plant genera, *Ipomopsis* and *Mertensia* based on pollen morphology. *I. aggregata* is a long, tubular flower almost exclusively pollinated by hummingbirds (28). This species has a large elevational range with phenology varying by elevation (29). Likewise, both *Mertensia* species have short open flowers (*M. fusiformis* and *M. ciliata*), but each varies in elevational range and the shorter *M. fusiformis* is more common in June, whereas the taller *M. ciliata* is more common in July (30). To prepare the Fuchsin gel, we added 50 grams of gelatin to 175cc of distilled water in a beaker and heated until the mixture dissolved. Next, we added 150cc of glycerin and 50 grams of crystalline phenol. We added as many crystalline basic fuchsin gels as necessary in order to create the desired color of the gel (31). Binary traits like pollen presence/absence were treated separately and coded as 0 or 1 for the species *Ipomopsis aggregata* and the two species in the genus *Mertensia*. While also found in Gunnison County, *I. tenuituba* was discounted as a source of pollen as it is used principally by hawkmoths and is not found in our study sites (32). We were unable to discern species of *Mertensia*, but each has a similar floral morphology and divergent flowering phenology, so are treated as a single flower type (30).

We visualized the distribution for all phenotypes of bill morphology in addition to wing chord as a measure of body size in `ggplot2` and calculated summary statistics for each raw and transformed phenotype by sex (33). Then we performed a principal component analysis on different subsets of traits due to variable missingness to assess phenotypic covariance between traits. We scaled all phenotypes before input and included transformed phenotypes instead of raw values if transformations were required in the following variance control steps. Principal components were then visualized with `pca3d` in R (Figure 1; Supplementary Figure 1). We

alternatively displayed the correlations between traits as a heat matrix using ggplot2 (Supplementary Figure 6).

Modelling Pollen Prevalence

We first wanted to determine which abiotic covariates are important for predicted pollen presence or absence. For these reasons, all modelling was performed on samples collected in Gothic as this site represented the largest sample size, has a well-known phenology, and has both species common and present annually. A logistic regression with a binomial logit link was implemented for each pollen type including the following covariates: day of year, sex of bird sampled, year as a factor, elevation as numeric, elevation as a factor, and month of sampling. We then ran the step function on each model to find the best fit to the model by iteratively dropping terms.

We then wanted to test whether bill morphology is a good predictor of pollen prevalence in the context of flower shape. We included three phenotypes as predictors of pollen prevalence: exposed culmen, culmen width, and curvature. Interactions with flower species are expected to change throughout the growing season as the floral community composition predictably waxes and wanes. To study this, we also included an interaction effect with day of year for each phenotype covariate. We ran the step function and a type III ANOVA on the reduced model including the relevant abiotic covariates. We used these models for the entire subalpine flowering season, where *Ipomopsis* and *Mertensia* abundance varies over time (32). We plotted a logistic function for the presence of each pollen type as a function of bill length and DoY in ggplot2 using the raw data for Gothic, CO. We also plotted the marginal mean predictions for *Mertensia* and *Ipomopsis* pollen prevalence as a function of DoY or exposed culmen length along with ROC curves to evaluate the sensitivity of each model.

The composition of species in flower communities changes over the course of the growing season. Optimal foraging theory predicts that if bill traits are directly influenced by flower morphology. As bill length and curvature have been linked to usage of important floral resources, we predict that the mean or variance of bill size and shape of sampled birds should shift between the early and late summer. We first ran a linear mixed-effects model where the response variable was either the raw or transformed phenotype for the Gothic dataset. Day of year was included in these models as a covariate along with year as a random effect to control for annual variation. We then created a new variable that binned all data from May and June as “early” and July and August as “late” and included this as a covariate along with all relevant interaction effects. We assessed these models with a backwards step function and a type III ANOVA. The fit of each model was then visualized using a qqplot of the model residuals and residual standard error.

Capture/Recapture Data

All instances of capture over the course of 2015-2019 were recorded, including the date of first capture and all instances of recapture within and between growing seasons. Many individuals were first captured and banded prior to this study prior to 2015, but were sampled for genome sequencing. Across the course of this study, 313 birds were recaptured at least once, representing ~50% of the total sample size. In these cases, their original banding date was used as the date of first capture. All dates, including date of first capture and any recapture dates, were converted into a ‘day of year’ (DoY) variable that could be used for comparison of capture date between growing seasons. We then plotted a distribution for the DoY for first capture date of all

birds banded in Gothic or Almont. We limited this analysis to these sites because our largest banding efforts took place at these sites. Next, we wanted to determine the extent to which birds were recaptured within the same interval during the growing season in each year. For each bird that was recaptured between growing seasons, we calculated the average difference in DoY between each pairwise DoY using only the earliest instance for each growing season. We then plotted this distribution and reported the mean and standard deviations of average differences (Figure 4)

Genome-Wide Association and Genetic Correlation Analyses

We tested for genotype-phenotype associations using a univariate linear mixed modelling (LMM) approach with GEMMA (0.98.3) (34). Our sample set contained a gradient of low to high relatedness between individuals, and an LMM approach allowed us to control for relatedness between individuals as a random effect. We first converted our imputed VCF for the entire sample to a mean genotype format (bimbam) using ngsQC (35). Then we filtered out genotypes from unplaced scaffolds, and chromosomes 4 and 32 to compute a relatedness matrix as input for the next step. An association test was performed for all phenotypes along with each respective covariate file on the LD-pruned and total SNP set for variants with a MAF greater than or equal to 0.05. The SNP-based or chip heritability is the proportion of variance in phenotypes explained (PVE) by additive genetic variation, a measure of heritability in the narrow sense, and was calculated along with p-values for the likelihood ratio test statistic at each SNP. P-values from the likelihood ratio test were extracted and converted to a chi-square density with one degree of freedom in R. Missing or infinite test statistics were then removed and p-values were adjusted with a false discovery rate of 5%. All adjusted p-values with a $-\log_{10}$ transformation were displayed in a Manhattan plot using the qqman package (36).

Several genes have been implicated to be associated with beak shape, but little is known about the amount of shared genetic architecture between quantitative bill traits. We began to assess the covariance between beak traits by visualizing the principal components of different pairs of phenotypes and their respective loadings in R. We then estimated the genetic correlations between traits to determine if phenotypic covariance corresponds to the amount of genetic covariance. We fit a multivariate linear mixed model in GEMMA (v0.98) to test for associations between pairs of phenotypes, simultaneously controlling for relatedness among samples in the LD-pruned SNP set. All runs with a large number of hits were subjected to a GO enrichment analysis in the Shiny GO system web interface (37). All results were plotted as they were for the univariate method.

Narrow Sense Heritability and Genomic Architecture Analyses

Linear mixed models assume that all SNPs have normally distributed effects on the trait of interest (38). This allows LMMs to be accurate estimators of SNP heritability when a large proportion of SNPs have an effect, but results in a lack of precision for traits with few genes of large effect. Bayesian sparse linear mixed models (BSLMM) use MCMC to estimate the proportion of phenotypic variance explained by variants (PVE) and the proportion of genetic variance explained by variants of major effect (PGE). This is achieved by assuming a mixture of two normal distributions of effect sizes, while controlling for relatedness (34). This in effect allows for the direct inference of genetic architecture by assigning variance explained by genes with sparse effects and genes with additional effects. We performed a BSLMM on all traits of interest using the residual phenotypes as input (See *Covariates*). All traits were run using the

LD-pruned and total SNP set to quantify the overestimation of heritability due to LD. From the BSLMM output we determine the approximation of phenotypic variance explained by variants (PVE). The genetic component of heritability was reported in two parts as the proportion of genetic variance explained by variants of major effect (PGE) as well as the sparse component of total genetic variance (ρ). We also reported the proportion of variants with nonzero effects (π), the number of variants with major effects (γ), highest sparse effect sizes, and those with the highest probability of having a large effect on traits as measured by the posterior inclusion probability (PIP). The distribution of candidate SNPs of large effect across the genome were visualized by plotting SNPs with the highest PIP and sizing points by their effect sizes.

A first pass at predicting the function of causal or linked SNPs is to annotate gene sequence around each candidate SNP. We first converted the position of each candidate SNP into bed format using bedtools then intersected the position with the overlapping or closest predicted gene from the GFF file with bedtools closest (39). Each gene with a predicted protein sequence from (40) was included as a query against the entire UniProt database using a high throughput blastp search with Diamond (v2.0.14) (41, 42). The output was limited to the protein with the highest score from the UniProt database and the reference taxa from which the protein was annotated.

Assessment of Shared Genomic Architecture

We wanted to assess the interactive genetic architecture underlying phenology, morphology, and behavior by quantifying genetic correlations between foraging phenotypes. Candidate SNPs associated with bill length (exposed culmen or full gape) or DoY were used to test whether SNP effects were shared between both foraging traits. We included these candidate SNPs in a multivariate LMM GWAS to look for genetic variance shared between bill length, arrival time, and *Ipomopsis* pollen prevalence. We used pollen prevalence data only from Gothic and Almont as this included both regions where *Ipomopsis* was present with a known phenology and additionally did not perform this analysis with *Mertensia* as we could not distinguish between the early versus late species using pollen morphology. SNPs identified as being genetically correlated between these three traits were identified using an *fdr*-corrected critical value of 0.05. We then input a list of overlapping or nearest gene for each SNP to the Shiny GO pipeline for gene enrichment analysis (37). Genetic correlations were reported by dividing the covariance between each trait pair by the square root of the product of genetic variances for each trait. The relative effect of SNPs on each trait was then determined by taking the average effect sizes from each candidate SNP on each phenotype and then dividing by the summed average. Case-control status for pollen GWAS were assigned binary values of 0 and 1.

Pearson correlation coefficients between expected genotypes were calculated for each pair of candidate SNPs from the full set of bill length or arrival associated SNPs as well as those identified in the multivariate LMM with *Ipomopsis* using ngsLD. We then calculated the LD for a random subset of SNPs by taking every 10Kth SNP from the filtered set for a total of 3185 SNPs. We reported the average and sample standard deviation for the Pearson correlation of SNPs with a MAF greater than or equal to 5% for the genomic background, candidate SNP set, and candidate SNPs passing an FDR-corrected critical threshold for genetic correlation between length, arrival, and pollen prevalence. LD decays around 10Kb, so any candidate SNPs within 10Kb of one another were pruned and one SNP was randomly selected. Finally, all SNP pairs with values exceeding two standard deviations of the genomic background were reported.

Results

Phenotype Analysis

Phenotype information was collected from 510 resequenced BTH individuals, including 261 females and 259 males, across 14 sites in four distinct regions of Gunnison and Delta county, Colorado (**Error! Reference source not found.**). The majority of bill traits represented a metric of bill size such as length, depth, or width where the mean value for females was shifted upwards relative to males (Supplementary Figure 1). Bill curvature did not have units with respect to size, but rather described an aspect of bill shape where the trait distribution for males and females was mostly overlapping. In contrast, the ratio of bill length to width, which also has no units, was shifted to higher values in males than females. Female values for wing chord, a metric of body size, had a slightly higher mean than that of males, but were mostly overlapping in distribution. Counts of iridescent throat feathers were only conducted in females and were highly zero-inflated with a mean of around four gorget feathers among birds that had them.

Shape information could be compared independently of bill size metrics from a dorsal and profile perspective, in both cases having been converted into principal components (Figure 1; Supplementary Figures 2-5). The first principal component of dorsal shape represented 68.8% of dorsal shape variation and described variability in the bill width to length ratio from the culmen base to the bill tip (Supplementary Figure 2,5). Dorsal variation along principal component 2 (14.8%) further described variability in a subtle constriction towards the center of bill. The first principal component of profile shape, in contrast, explained 53.6% of profile shape variation, where constriction toward the center of the bill varied through the ratio of bill depth to length (Supplementary Figure 2,4). The relative distance of the bulge at the bill tip from the culmen base was described by the second principal component (21.5%). Comparisons of the mean dorsal and profile shape between males and females showed only slight differences in shape independent of size but were significantly different for dorsal shape PC1, and profile shape PCs 1-2 (See Covariates) (Supplementary Figure 3).

Many of the bill phenotypes positively covaried with one another while others varied somewhat independently (Supplementary Figure 6). Unsurprisingly, both length metrics and both width metrics covaried with one another. Notably, the first principal components of dorsal and profile shape positively covaried with one another, while their second components varied independently. This resulted in higher width to length ratios corresponding to a higher depth to length ratios. Variability in shape also covaried with certain metrics of size. For example, a higher width to length ratio represented a wider bill. Bills that were less constricted towards the center of the bill with a higher depth to length ratio (profile PC1) were also shorter, wider, and deeper resulting in a bulkier bill. Curvature, however, did not correlate strongly with any traits and explained why variability in this trait had loadings on a separate axis of principal component variation (Figure 2). Generally in birds, body size usually covaries with metrics of bill size, but wing chord was only weakly correlated with any one trait in the BTH and varied mostly independently (43). Traits that were not normally distributed in this analysis were first transformed using the *bestNormalize* package in R to reduce the disproportionate influence of extreme high or low values (44).

Pollen prevalence was used as a proxy for visitation of two morphologically distinct flower species. These data were used to test if heritable variation in foraging traits was associated with the probability of pollen presence or absence. This provided a glimpse into whether there is evidence of a bill-to-flower shape correspondence at the population level. Models of phenotype data over the course of the entire season were performed to determine general trends for

predicting the use of *Ipomopsis*, a long, tubular hummingbird-pollinated flower. We found that day of year and culmen length were positively associated with *Ipomopsis* pollen along with an interaction term of culmen length by day of the season (Figure 4; Supplementary Table 4).

We contrasted these results with the presence of *Mertensia*, which was representative of two species with short, open flowers, one of which is common in the early summer and the other in late summer (Figure 4). Models of *Mertensia* pollen incidence across the entire summer reported associations with culmen length, culmen curvature, day of year, and interactions of day of year with curvature (**Error! Reference source not found.**). Overall trends showed that straighter bills were associated with higher probability of *Mertensia* spp. pollen presence across the entirety of the summer (Supplementary Figure 6). Exposed culmen length, however, was negatively associated with *Mertensia* pollen presence over the course of the summer. This revealed birds with longer bills had a higher probability of harboring pollen from *Ipomopsis*, while those with shorter bills had a higher prevalence of *Mertensia* pollen.

Birds may be foraging in regions where they can feed most efficiently from the local flora, and feeding efficiency is linked to bill shape (45). Therefore, we tested whether the average shape or size for various bill phenotypes changed across the summer. Full gape and culmen length were the only two phenotypes detected to have a shift in the mean value over the course of the summer (Figure 4). Variation across the summer in culmen width and the culmen length to width ratio were all explained primarily by sex differences whereas curvature, culmen depth, and PC1 of profile shape primarily varied annually. Gape width was explained by both annual variation and sex, while PC1 of dorsal shape did not vary annually, seasonally, or by sex.

Downstream genotype-phenotype analyses required controlling for covariates with phenotypes of interest to prevent associations with correlated phenotypes. Wing chord and sex were both significant predictors of exposed culmen and full gape, but wing chord had only a weak positive association with both metrics of bill length ($r=0.06$) (**Error! Reference source not found.**). Culmen width had only a marginal association with wing chord length, but a strong effect of sex, whereas gape width was associated with both. However, the ratio of culmen length to width was positively associated with both wing chord length and sex. Curvature also included sex as a significant covariate but was marginally associated with culmen length. Culmen depth, wing chord, PC1 of dorsal shape, PC1 of profile shape, and PC2 of profile shape were all associated with sex. PC2 of dorsal shape was the only phenotype with no association to wing chord length or sex. A tabular summary of significant covariates and normalization transformations are listed in **Error! Reference source not found.**

Capture/Recapture Data

Between Almont and Gothic, 469 birds were first captured between 2016 and 2019. The date of first capture was bimodal for Almont and Gothic and for each site individually a mean capture DoY of 178. The standard deviation in capture day was ~19 days and the difference in capture day within one, two, and three standard deviations was 38, 76, and 114 days respectively. We plotted the distribution of average difference in capture days between the day of year for first capture and day of year for the first recapture in subsequent years (Figure 5). Of the 469 total birds captured across years, 212 of these were recaptured across multiple seasons in Almont and Gothic, representing 67% of all birds that were recaptured within or between seasons (Figure 5). The mean difference in earliest capture dates between years was ~25 days with a standard

deviation of 16 days, conservatively representing around half the range in capture times within two standard deviations of capture dates.

Structure of Genotype Data

Allele frequencies can be inflated due to high relatedness, creating an increased risk for false positive rates in GWAS, or site frequency spectra that don't accurately reflect population history. In order to determine the influence of cryptic relatedness on our dataset, we first estimated the inbreeding coefficients of each individual using every 50th SNP, resulting in 637,153 sites. The mean inbreeding coefficient was 0.03 with a maximum of 0.23 (Supplementary Figure 11). These values were low enough that they did not need to be conditioned on in generating the folded site frequency spectrum. We used the same filters from the previous step to plot the folded site frequency spectrum across all sites using autosomal SNPs (excluding Chr 32) and see the expected exponential decrease in allele frequency bins (Supplementary Figure 12). Using the pairwise theta estimated from the site frequency spectrum and the germline mutation rate from birds, we solved for the effective population size giving us an estimate of 63,012.

Population structure can result in the inflation of summary statistics in GWAS, but can be visualized in a principal component analysis to determine whether additional filters are needed. To avoid this issue, PCA plots were generated for SNPs with a MAF of 1%, 5%, and 10% colored by sex (Supplemental Figure 13). For SNPs with a MAF of 1% or higher, we also color points by library prep kit, sequencer, and coverage. The first principal component of genotype likelihoods for all MAF cutoffs ranged from 1.05-1.64%, while the second and third principal components ranged from 0.37-0.65% and 0.36-0.64%, respectively. In all cases, there is a single cluster without evidence for population structure or sex-specific mapping problems. We additionally did not find structure in the data from library preparation kit, sequencer, or genome-wide coverage. However, structure in principal component analysis can arise from cryptic relatedness. Pairwise relatedness was calculated for each pair of individuals found at the same site and was plot as a histogram (Supplementary Figure 14). The mean relatedness among sample pairs from the same sites was ~0 with a maximum of 0.55 corresponding to a first degree relative pair such as parents and offspring or a sibling pair. This metric also allowed us to control for pairwise relatedness in estimates of average bill shape change over the summer. A mantel test using a distance matrix for day of year as the predictor variable and the relatedness matrix as a response variable, produced no significant effect of relatedness on time of capture or vice versa (r : 0.0001126, p -value: 0.42657).

Results of Univariate Linear Mixed Model GWAS

GWAS were run on each phenotype with all relevant covariates (See Covariates) and a MAF cutoff of 5%, 10%, or 20% for the LD-pruned and unpruned dataset. As a control, GWAS were also run on each untransformed and transformed phenotype with only sex as a covariate. SNPs from only a subset of phenotypes passed the FDR-corrected threshold and tended to reflect dimensions of the culmen base (**Error! Reference source not found.**).

A total of 15 hits associated with sex were located on chromosome 4, which is the avian Z chromosome in our assembly (**Error! Reference source not found.**). Two loci associated with sex were located on chromosome 3, three on chromosome 32, and one on scaffold 90. In total, 6 loci were associated with sex that were not located on the Z chromosome.

We detected a single candidate for the following bill phenotypes: culmen width, gape width, culmen depth, culmen length to width ratio, and bill curvature, with an additional two for principal component 1 of dorsal shape. A single hit outside of chromosome 4 associated with sex, *AGBR3*, associated with dendrite formation and temperament.

Estimates of percent variance explained (PVE) by all loci never differed by >10% based on the filters used (Supplementary Table 10). However, these differences were only apparent when covariates like body size were removed. Culmen depth, dorsal shape PC2, and profile shape PC2 had low PVE compared to the other phenotypes. Profile shape PC1, wing chord, day of year, and culmen width had moderate heritability between 20-40%, whereas gape width, culmen L/W ratio, and full gape had heritability between 40-60%. The highest heritabilities were measured for curvature and dorsal shape PC1 between 60-80%. The highest heritability using LMM was measured for counts of iridescent feathers, which was 99% heritable, a likely artifact suggesting the genomic architecture of this trait is oligogenic, and BSLMM is a more appropriate model (See *Results of Bayesian Sparse Linear Mixed Models*).

Results of Multivariate Linear Mixed Models

The degree of genetic correlation between pairs of phenotypes estimated with multivariate GWAS varied widely. Culmen depth, which had a low percent variance explained by SNPs in the univariate GWAS, did not show evidence of measurable genetic correlation with exposed culmen. Gape width x culmen width and culmen width x exposed culmen were weakly genetically correlated with $-0.05 < r < 0$. Profile shape PC1 x curvature, culmen width x wing chord, profile shape PC1 x exposed culmen, profile shape PC1 x culmen L/W, and dorsal shape PC1 x culmen L/W had stronger negative correlations between $-0.57 < r < -0.27$. The strongest negative correlation was reported between profile shape PC1 and full gape at $r \sim -0.99$. In the opposite direction, culmen depth x profile shape PC1, and culmen width x culmen depth were weakly genetically correlated $0 < r < 0.0002$, while curvature x F-curvature, curvature x full gape, full gape x wing chord, gape width x dorsal shape PC1, and culmen width x full gape had more moderate genetic correlations between $0.14 < r < 0.44$. The strongest positive correlations were between dorsal shape PC1 x profile shape PC1 and exposed culmen x full gape both of which had r values above 0.99.

Multivariate GWAS between full gape and exposed culmen identified 43 SNPs that passed the FDR-corrected critical threshold (Figure 3). The closest or overlapping annotated protein for each identified SNP associated with bill length was entered into the Shiny GO pipeline for gene ontology enrichment where the largest fold enrichment was suckling behavior. A single hit, *CAD17*, which is involved in calcium-dependent cell-cell adhesion, was identified in the multivariate LMM between profile shape PC1 and full gape. Two hits were identified as genetically correlated between culmen depth and profile shape PC1 in *S12A7* and *LRAT1*, which are involved in chloride ion homeostasis and cell morphogenesis, respectively. Nine SNPs in 8 different genes largely involved in neurogenesis were identified in multivariate GWAS between dorsal shape PC1, profile shape PC1, or culmen L/W. All genetic correlation estimates and candidate SNPs identified in multivariate LMM GWAS are listed in Supplementary Tables 11-12.

Results of Bayesian Sparse Linear Mixed Models

A separate measure of heritability including low MAF SNPs was also estimated under the assumption of two different distributions of effect sizes to determine the genetic architecture of each trait. The percent variance explained, or heritability of each trait varied widely as in the linear mixed model framework. The percent of phenotypic variance explained by all SNPs ranged from 28-39% for traits such as profile shape PC1, wing chord, culmen depth, or culmen width. Traits such as F-curvature, full gape, exposed culmen, culmen length to width ratio, gape width, and time of arrival had more moderate PVE estimates between 40-59%. The highest PVE estimates were found for curvature, dorsal shape PC1, and iridescent feather counts in females between 60-70% PVE (Supplementary Table 13).

The parameter ρ defines the proportion of genetic variance explained by sparse effects terms and ranges between 0 and 1, where lower values are indicative of a polygenic architecture, and higher values of an oligogenic one. ρ values were lowest for curvature, dorsal shape PC1, exposed culmen, F-curvature, and full gape with values between 20-30%, suggesting a highly polygenic architecture. Culmen width, wing chord, culmen length:width ratio, and iridescent feather count in females were slightly higher with ρ between 30-40%. ρ was around 50% for gape width and profile shape PC1 with roughly an equal mixture of polygenic and sparse architecture, while the highest values of ρ were found for time of arrival and bill depth. The estimated number of sparse effects variants were highest (>60) for both measures of curvature, full gape, profile shape, and wing chord. Culmen depth, feather count, and culmen length:width were lower between 26-50 variants while the sparsest effects were found for culmen width, dorsal shape PC1, exposed culmen, gape width, and time of arrival at fewer than 25 variants.

All BSLMM were run on an LD-pruned set and full set of SNPs (a list of all candidate SNPs can be found in Supplementary Table 14). PVE did not differ by more than 5% for most phenotypes based on LD-pruning. However, gape width PVE decreased after LD-pruning by 7% and increased by 7% for profile shape PC1. The most significant change after pruning was seen for time of arrival which showed an increase from 40%-75% PVE following LD-pruning.

We also reported discrepancies in heritability estimates between LMM and BSLMM since LMM assumes a polygenic basis for traits where all variants affect the phenotype. Because of this, LMM is less accurate at predicting heritability when there is a mixture of small and large effect size distributions, creating discrepancies in estimates of heritability between LMM and BSLMM. Furthermore, a MAF cutoff of 5% was implemented for LMM while all SNPs with MAF of at least 1% were included in BSLMM. PVE estimates ranged between 10-40% lower in BSLMM compared to LMM for feather count and dorsal shape PC1 due to the large contribution of sparse effects and an incorrect assumption of polygenic architecture in LMM. PVE was 10-40% higher in BSLMM for profile shape PC1, culmen depth, and time of arrival due to the missing heritability from low frequency variants below 5% MAF. We reported SNPs identified as being in the top .01% of nonzero effect sizes as well as SNPs estimated to have a sparse effect in at least 10% of MCMC chains. All SNPs identified with a PIP greater than or equal to 10% also overlapped with those estimated to have nonzero effect sizes, with the exception of 4 genes identified as having sparse, or large effects for time of arrival. Nine genes were identified as having sparse effects in BSLMM in association DoY, having the largest effect sizes of any sparse effects across GWAS for all traits (Figure 5).

In addition to multivariate LMM between traits, some genes were identified in multiple GWAS for different phenotypes. DMD, involved in actin cytoskeleton organization, was identified in GWAS for culmen depth and width. GP143 was reported in BSLMM GWAS for full gape and exposed culmen and is involved in calcium mediated signaling. PHEX was found

in association with wing chord and culmen width and is a mediator of bone development. Putative viral polymerase genes were also identified for culmen length:width, exposed culmen, and wing chord and are involved in DNA integration and recombination. RERGL was associated with two shape phenotypes, F-curvature and culmen length: width, and is a G-protein activator involved in GTP-binding. BROX, expressed in extracellular exosomes, was found in association with dorsal shape PC1 and full gape. LRAT1 was associated with the largest number of phenotypes: culmen depth, dorsal and profile shape PC1, and culmen length: width, and is related to cell morphogenesis and motility. Lastly, SMCA1 is involved in brain development and neuron differentiation and was identified in GWAS for exposed culmen and gape width.

Assessment of Shared Genomic Architecture

A multivariate GWAS run on *Ipomopsis* pollen prevalence, bill length, and DoY included a panel of 106 SNPs identified in GWAS for bill length and DoY. From this SNP panel, forty-four genes were identified as having passed the *fdr*-corrected *p*-value threshold $-\log_{10}(0.05)$ for having shared variance between all three traits. GO terms were enriched for locomotory exploration behavior, mating behavior, and chemical synaptic transmission, particularly with respect to signaling and cell-cell communication (Figure 6). *Ipomopsis* pollen prevalence had little genetic correlation with either bill length or arrival time between -0.002 and -0.004 respectively. The majority of genetic correlation was found between arrival time and bill length with $r=0.46$.

The Pearson correlation between expected genotypes as a metric of LD had a mean of 0.02 for the list of SNPs associated with length and arrival with a standard deviation of 0.01. This matched the genome-background null sample, which also had an average LD of 0.02 with a standard deviation of 0.01. For SNPs that passed the critical threshold in the MLM for arrival time, length, and *Ipomopsis* presence, average LD was similar, but had a right skewed tail with an SD of 0.03 in the candidate SNP set. 27 SNPs were identified in the right-skewed tail as being above the 95th percentile of pairwise LD values. SNPs with long-range LD were then subset to include only those which were found on multiple chromosomes ($N=14$) and at least 2Mb apart ($N=14$). SNPs above the 95th percentile are listed in Supplementary Table 16. We assessed annotated SNPs for those overlapping genes with many known pleiotropic functions ($N=7$) and included these in a gene ontology enrichment analysis using the ShinyGo pipeline.

Discussion

Optimal foraging theory dictates that natural selection should optimize the efficiency of pollen transfer in plants and nectar use by their animal pollinators (1). Such traits often manifest as ‘syndromes’ or ‘ecotypes’ that covary in a functionally integrated adaptive fashion, and are thus expected to be correlated to some degree at the genetic level, resulting in adaptive pleiotropy (46). The genomic architecture of pollination syndromes in plants has been well-characterized, suggesting some degree of shared and unique genetic underpinnings of between floral traits involved in the partitioning of pollinator types (7) as well as the ecological drivers of this trait variation (47). However, little is known of the link between foraging traits and their underlying genomic architecture in animal pollinators. Here, we provide an animal pollinator’s perspective of the relationship between morphology and behavior in the context of ecology, and the underlying interactive genomic architecture of this relationship. Specifically, we illustrate the interaction between linked variation in floral preferences, bill morphology, and long-distance

migration phenologies in a population of the Broad-tailed hummingbirds at the phenotypic and genomic levels and propose a model for subtle intraspecies niche differentiation.

The BTH has been a model system for pollination ecology, plant-pollinator co-evolution, and eco-physiology. More recently, because it is a Neotropical migrant dependent on ephemeral nectar plants that exhibit predictable flowering phenologies in its breeding range, it has been used to study the impacts of climate change on pollinator foraging behavior. Early snow melt followed by damaging frosts is a feature of anthropogenic climate change in Gothic, Colorado, and has produced a mismatch between arrival time of the BTH and the availability of major nectar plants like scarlet gilia. Thus, a major question is whether heritable variation for foraging traits exists in the BTH under a regime of rapid environmental change that centers on flowering phenologies of the nectar plants.

To begin to address this question, we focused on three sets of traits: bill morphology, prevalence of bill pollen from two important nectar plants, and yearly arrival date, using recapture dates. We found substantial heritable variation in each of these traits. This variation was highly heritable and explained by both oligogenic and polygenic architectures. GWAS for bill morphology and migration phenology identified large effect genes with known functions important for migratory behavior and bill morphological development. The genomic architecture of multiple traits involved in foraging were found to be partially overlapping. We found shared effects between phenotypes from candidate SNPs and LD above background for genes with known pleiotropic effects in behavior and morphology.

Overall, the generally predictable local flowering phenology covaries with variation in local migration timing of the BTH, which is highly heritable. Second, heritable bill length variation is associated with both the local flowering phenology and the timing of local migration where both bill length and curvature are linked to the probability of pollen prevalence in an atypical and ornithophilous flower.

Flowering Phenology Predicts Local Migration

BTH individuals migrate to breeding sites, largely in the Cordillera of the U.S. in the Spring from Central America. The timing of each migratory segment of northward flight is correlated with nectar availability at the breeding sites (8). Two exclusively hummingbird-pollinated flowers co-occur in the BTH nesting habitat, *I. aggregata* and *Castilleja linariifolia*, and emerge in late summer where they compete for pollen visitation (48). Both flowers are typical of ornithophilous species, producing large nectar rewards with long tubular corollas, and are well studied in the context of BTH pollination biology (19, 49). Other atypical flowers are available throughout the summer, including two species of *Mertensia*, an early flowering species, *M. fusiformis*, and a late flowering species, *M. ciliata*, both of which are frequented by the BTH (12, 30).

Initial capture dates ranged from early June to late July across this 5-year study with two peaks in initial capture timing in mid-June and mid-July, where the second wave of arrivals corresponded to an overlap with both ornithophilous flower species. Of birds recaptured between seasons, individuals were on average caught in the same three week interval between years, consistent with an early or late-wave arrival. These data comported with the fact that individual BTHs display strong philopatry to past breeding sites (50). The predictable flowering phenologies at the breeding sites, until very recently, may give earlier waves of migrants access to primarily atypical flora shared with bumblebee pollinators and waves of later arrivals may have more restricted access to hummingbird-specialized species with longer corollas. This

scenario dovetails with our next set of observations on bill traits, which co-varied with arrival times and, remarkably, genetic correlations were present between architectures associated with variation in migration phenology, bill length, and pollen prevalence. Candidate SNPs provided evidence for inter-chromosomal LD of genes with pleiotropic effects relevant to foraging, likely resulting from coselection on coadapted trait combinations.

Beak Length Predicts Pollen Prevalence on the Beak

The incredible match between the lengths and curvatures of hummingbirds and the corresponding structures of their specialized, nectar plants are among the most iconic examples of co-evolution. Nonetheless, many hummingbirds, including the bee hummingbirds that migrate into the higher latitudes of North America, consistently use flowers longer or shorter than their bill (10). Longer bills provide larger maximum extraction depths while shorter bills result in fewer insertion errors, giving each a relative advantage at long and short flowers, respectively (45). Additionally, trait matching between corolla and bill curvature permit a higher efficiency of nectar intake (51). Therefore, relative floral usage should differ between individuals that vary in bill length or curvature when mismatches occur.

We found an increased probability of pollen prevalence in both *I. aggregata* and *Mertensia* when trait matching between bill and flower was larger. Specifically, probability of *Ipomopsis* pollen presence increased on bills that were longer than their counterparts while the much shorter corollas of *Mertensia* were associated with shorter bills. If feeding efficiency is a function of this bill-flower correspondence, individual phenology of bill morphology may be linked to floral phenology.

Local Migration Phenology Predicts Beak Length

Increased tracking abilities in bird studies have revealed that considerable migration route and timing variation exists between individuals of the same species (52). Birds with variable migration phenotypes will experience different environments, where niche use is predictable (53). In some studies of long distance migrants, migration phenology covaries with bill length (54) and bill length is a predictor of diet (55). While variation exists for both local migration time and bill length in BTH, local migration time covaries with bill length such that birds arriving later have longer bills. Average corolla length increases over the course of the summer (56) and atypical flowers are available throughout the season in Gothic (10). These results, in the context of previous pollen prevalence analysis, imply that individuals arriving earlier encounter flora primarily with relatively short corollas (atypical flowers) such as *Mertensia*. In the late summer, variability in floral length should increase with the emergence of ornithophilous flora with long corollas like *I. aggregata* and *C. linariifolia* (19), at which time longer-billed birds have a higher probability of visiting long flowers and shorter-billed birds with shorter flowers, which they feed at more efficiently. These factors may promote resource partitioning or partial niche differentiation.

Genomic Architecture of Bill Morphology

Many candidate genes have been identified in the context of variation in bill shape and size in other birds, particularly in Darwin's Finches, among other passerines (15–17, 57). We also found that most of these candidate genes were associated with heritable variation in beak shape and size in the BTH. However, the largest effects identified were attributed to previously unidentified genes. While all traits had some degree of heritable genetic variation, including

subsets of large effect loci, phenotypes varied widely in heritability and genomic architectures. Some traits that were largely heritable and ecologically relevant in Darwin's Finches, such as bill depth, which is directly involved in the mechanics of seed cracking, showed the lowest heritability among bill traits in the BTH. While heritability varied between BTH and Darwin's Finches for bill depth, most of the existing heritability could be explained by large effect loci, which is consistent with other studies in birds showing this trait is primarily oligogenic (58). Likewise, bill length was mostly polygenic in the BTH, comporting with previous studies (15, 59–61). Our GWAS for bill length, the most polygenic and ecologically salient trait in the BTH, identified 91 loci significantly enriched for ion transmembrane transport and platelet activation, which are involved in bone growth and cell proliferation, respectively (62). The enrichment of small, additive effects related to bill length in the BTH is particularly relevant to the potential for local adaptation in this species as the same phenotype is polygenic in other birds, where polygenic adaptation has occurred in response food resource availability (15).

Genomic Architecture of Migratory Behavior

Other traits related to behavior were at the other end of the genomic architectural spectrum, with local migration time having the most oligogenic architecture of all traits we measured in the BTH. Narrow sense heritability was particularly high for this trait in the BTH (0.75) and at the same time, the genomic architecture underlying this heritable variation was largely oligogenic with an estimated 12 sparse effect variants. Heritability estimates between studies for arrival timing have varied widely in other bird species: the median narrow sense heritability from these studies is 0.301 and ranges from 0.07-0.67 (63).

Candidate genes that have received the most attention in the context of variation in migration phenology are involved in circadian rhythm and personality traits such as exploratory behavior, and are known to be involved in local adaptation to seasonal variation at different latitudes (64). The strongest effects for heritable variation in arrival time in the BTH were detected in loci involved in circadian clock (OPN5), aggressive behavior/aging (PENK), oxidative stress (ATP11A), and lipid metabolism (ACSS3, HAO1). Of particular interest is OPN5, which is involved in photoperiodic induction in birds (65), regulates retinal rhythm similar to that of cryptochromes using UV light (66), and is involved in intracellular calcium regulation (67). Across all genes, the largest enrichments for variation in arrival time were for ontology terms that included photoreceptor activity and hyaloid plexus regression, which are both involved in vision and development of the eye. These findings show that natural variation within a BTH population in Gothic, Colorado in both migration phenology and bill morphology have a largely heritable basis, and that both phenotypes covary predictably with flowering phenology. These potentially co-adapted behavioral and morphological traits may then be expected to have some degree of interactive genetic architecture—overlapping sets of causal loci or co-selected loci across the genome, or both.

Genomic Architectural Network of Foraging Traits

We found clear correlations between average arrival time, average bill length, and two important nectar plants as a proxy for general patterns in the floral community. This suggests the possibility that variation in foraging phenotypes overlap in the genomic architectural network. Large effect loci identified in association with arrival time and bill length revealed that genetic correlations, or the genetic variance shared between these two foraging traits, is high, suggestive of pleiotropy, coselection, or causal overlap. Furthermore, the interaction of these two

phenotypes with *Ipomopsis* use identified a subset of 45 loci with an effect between all three traits. The majority of genetic correlations were between variation in arrival time and variation in bill length and were enriched for genes involved in locomotory exploration behavior, mating behavior, and chemical signaling. The low genetic correlations between foraging traits and *Ipomopsis* prevalence is suggestive of the fact that this is but one focal plant representing a fraction of the available floral community likely involved in diffuse coevolution with bill traits.

Five candidate genes from those enriched for locomotory exploration behavior were identified as having long range contacts above the genomic background. These included TY3H involved in the dopamine biosynthetic process, and four other genes with multiple contacts: DCE1, ESR1, PENK, and GRM5. Glutamate receptor 5 (GRM5) was particularly pleiotropic with known functions in calcium-mediated signaling and taste memory, while others like DCE1 were involved in glutamate catabolism and locomotory exploration behavior. Other candidates PENK, involved in aggression, behavioral fear response, and aging, and ESR1 involved in the androgen metabolic process or estrogen stimulus, were identified further supporting the link between behavior and morphology, both primary aspects of foraging, at the genomic level.

Conclusion

Seasonal environments are characterized by temporally-varying resource availability, and are in addition spatially variable particularly for migratory species. Both are characteristic of the alpine meadows foraged by the BTH, where the vast diversity of floral species act as a substrate for natural selection to shape foraging traits. Generalist foragers like the BTH have likely been shaped by diffuse coevolution with a wide variety of food plants, where bill size and shape are inherited in a modular fashion via their unique genomic architectures. Such intra-species variability in morphology has likely facilitated optimal foraging behavior such that individuals preferentially visit flowers that match their bill morphology.

Pollen prevalence as a function of flowering phenology and bill morphology provide evidence for subtle resource partitioning, niche divergence, and ecological specialization between individuals. This seems to have been facilitated by covarying migration phenotypes and beak morphology. Such patterns are apparent in an interactive genomic architecture. This process may unfold through transient genetic correlations between traits, wherein a subset of genes affect networks of traits including both morphology and behavior, each of which is relevant to life history differences. Genetic correlations between foraging traits like bill morphology and migration phenology may break down over short timescales as reflected in the low genome-wide patterns of LD reflected in this species and may facilitate rapid adaptation in the face of environmental change. We expect the effects of climate change on the floral phenology will be particularly relevant to foraging adaptation in alpine species like the BTH, where a quickly advancing floral phenology may reduce the breadth of food resources available throughout the season. Long term research on this system will undoubtedly advance our understanding of how variation is maintained in the face of environmental change.

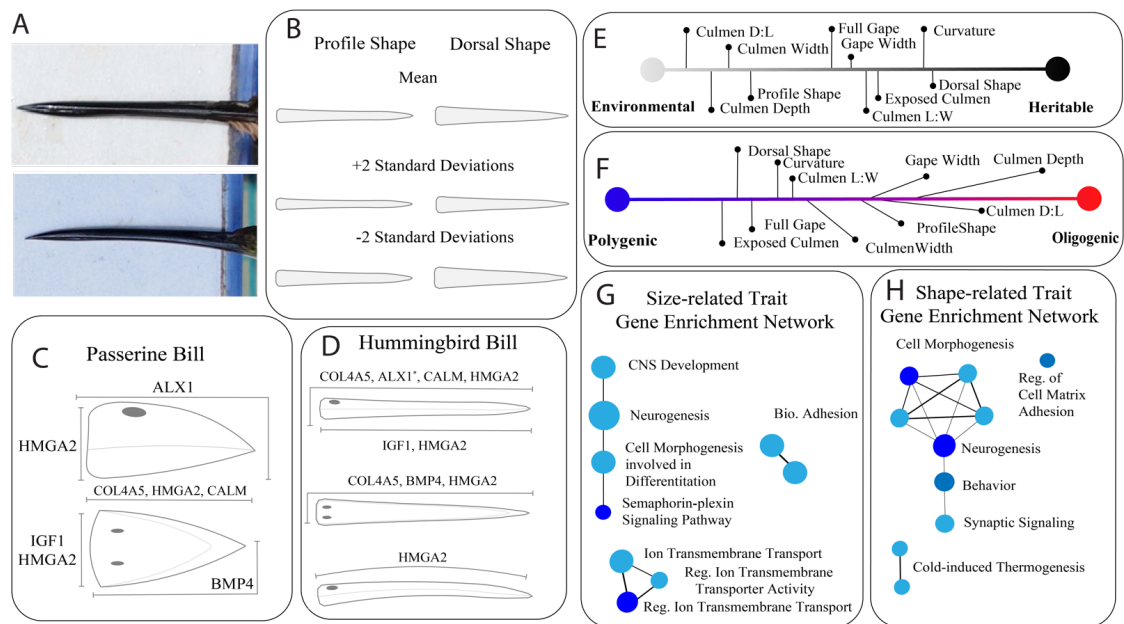


Figure 1. Summary of narrow sense heritabilities and genetic architectures of bill morphological traits in the BTH. A. Variability in length and curvature in two adult male BTH. Bill lengths are not to scale. B. Distribution of profile and dorsal shapes of bill outlines with asymmetrical and size variation removed. Profile shape primarily explains the depth-to-length ratio, while dorsal shape describes the width-to-length ratio. C. Genes identified in association with bill shape and size in Passerines. Lines denote the phenotypes associated with each gene of interest. D. Genes identified in association with bill shape and size in the BTH. All genes listed were within 20Kb of candidate genes, with the exception of ALX1 (65Kb). These genes did not represent the top effect sizes for each trait, but had significant effects. E. Scale depicting narrow-sense heritabilities of phenotypes measured in the BTH. All values were measured using BSLMM. F. Scale depicting the proportion of large effect alleles relative to all alleles with nonzero effects, a proxy of polygenic to oligogenic genomic architecture. G. Gene ontology enrichment network showing ontology terms enriched for traits with size (measured in millimeters). H. Gene ontology enrichment network showing ontology terms enriched for traits as a proxy for shape (unitless; independent of size).

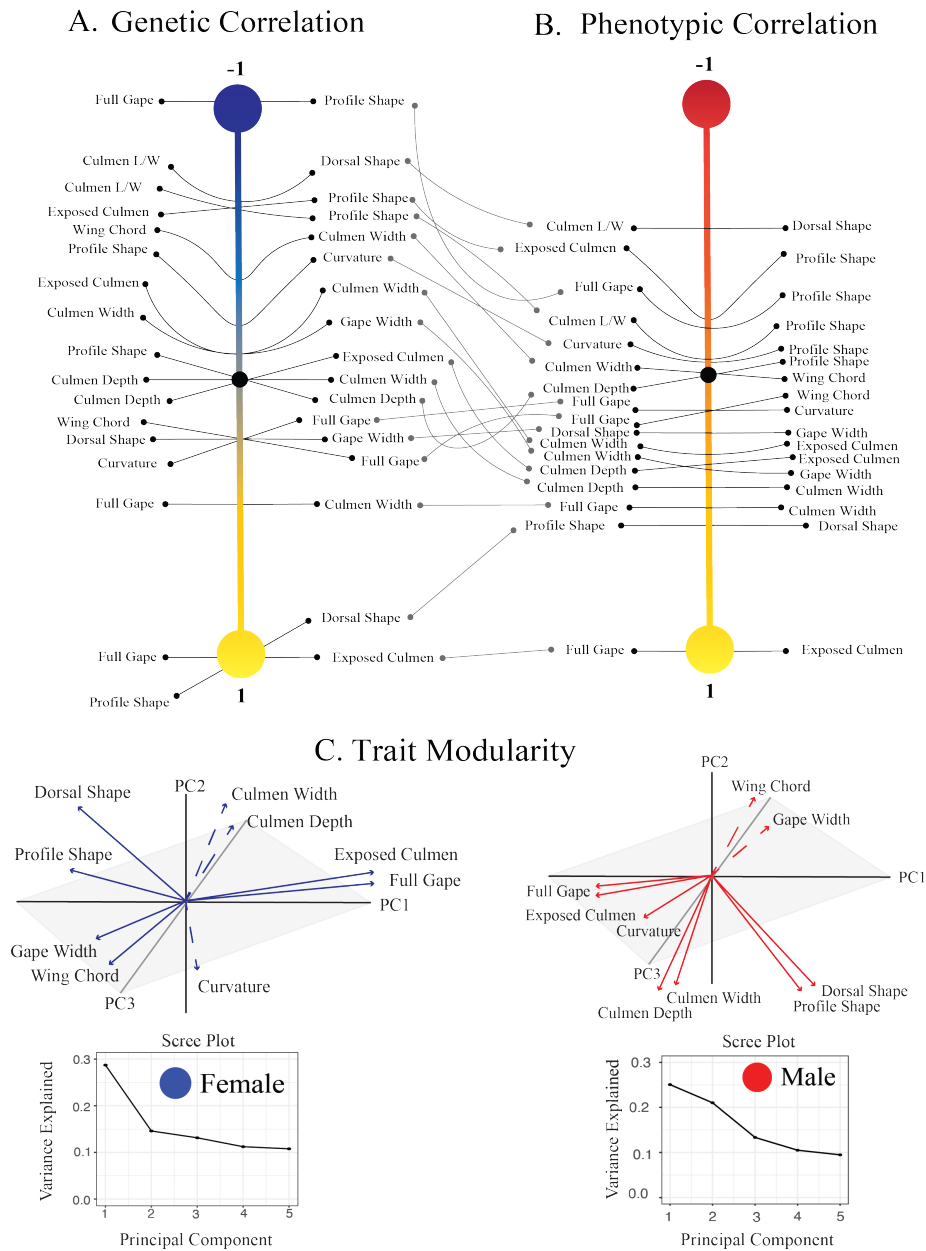


Figure 2. Summary of the relationship between phenotypes at the level of morphology and genetics. A. Scale displaying the genetic correlations between traits on a scale from -1 to 1. B. Scale displaying the phenotypic correlations between traits on a scale from -1 to 1. Panels A and B are connected with light gray lines to denote the correspondence between measures of phenotypic and genetic correlation. C. PCA of phenotypes in males and females separately with corresponding scree plots.

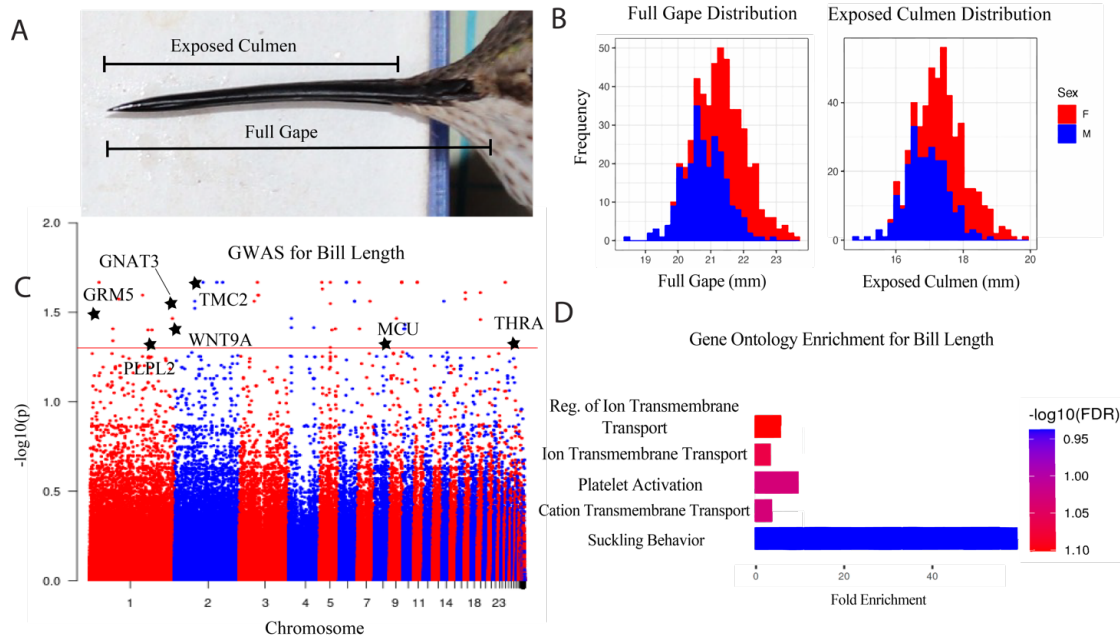


Figure 3. Summary of phenotypic and genomic variation for bill length. A. Bill length was measured using two different measurements, full gape and exposed culmen. Full gape is measured from the base of the mandible to the bill tip and exposed culmen is measured from the feathers on the maxilla to the bill tip. B. Full gape and exposed culmen measurements are normally distributed in this population. C. Multivariate GWAS for exposed culmen and full gape identified 43 loci that passed an FDR-corrected p-value threshold of $-\log_{10}(0.05)$. Stars denote loci of interest involved in calcium ion homeostasis. D. Gene ontology enrichment analysis shows regulation of ion transmembrane transport, involved in bone formation, and platelet activation, involved in cell proliferation, as the most significant terms. Total sample size for all analyses was 510; 261 females and 249 males.

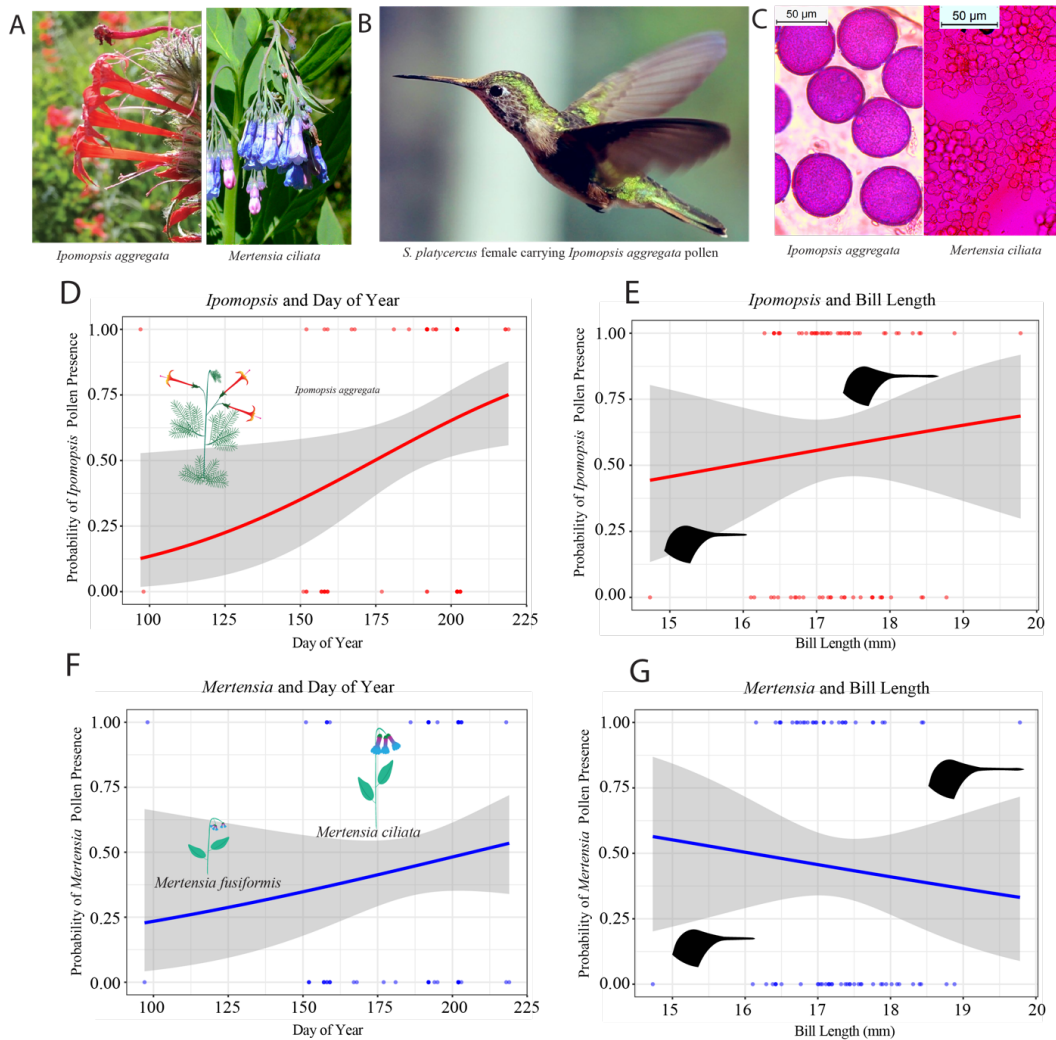


Figure 4. Bill length is linked to probability of pollen prevalence for two species of morphologically variable flowers. A. *Ipomopsis aggregata* (left) and *Mertensia ciliata* (right) are both frequented by the BTH. B. Adult BTH female with *I. aggregata* pollen along the length of the bill. C. Pollen from *I. aggregata* (left) and *Mertensia* spp. (right) were identified from bill swabs and were diagnostic via microscopy methods. D-G. Probability of pollen prevalence is a function of floral phenology and bill length. D. *I. aggregata* pollen prevalence probability increases in the late summer relative to the early summer, overlapping with its phenology ($p=0.002699$). E. Pollen prevalence probability of *I. aggregata* increases when bills are longer ($p=0.005458$). F. *Mertensia* spp. pollen prevalence probability is relatively constant over the course of the season, with two sequentially flowering species ($p=0.022133$). G. Pollen prevalence probability of *Mertensia* spp. decreases when bills are longer ($p=0.049504$). All plots depict logistic curves of raw data to represent trends identified in generalized linear mixed models. For marginal means, see Supplementary Figure 7.

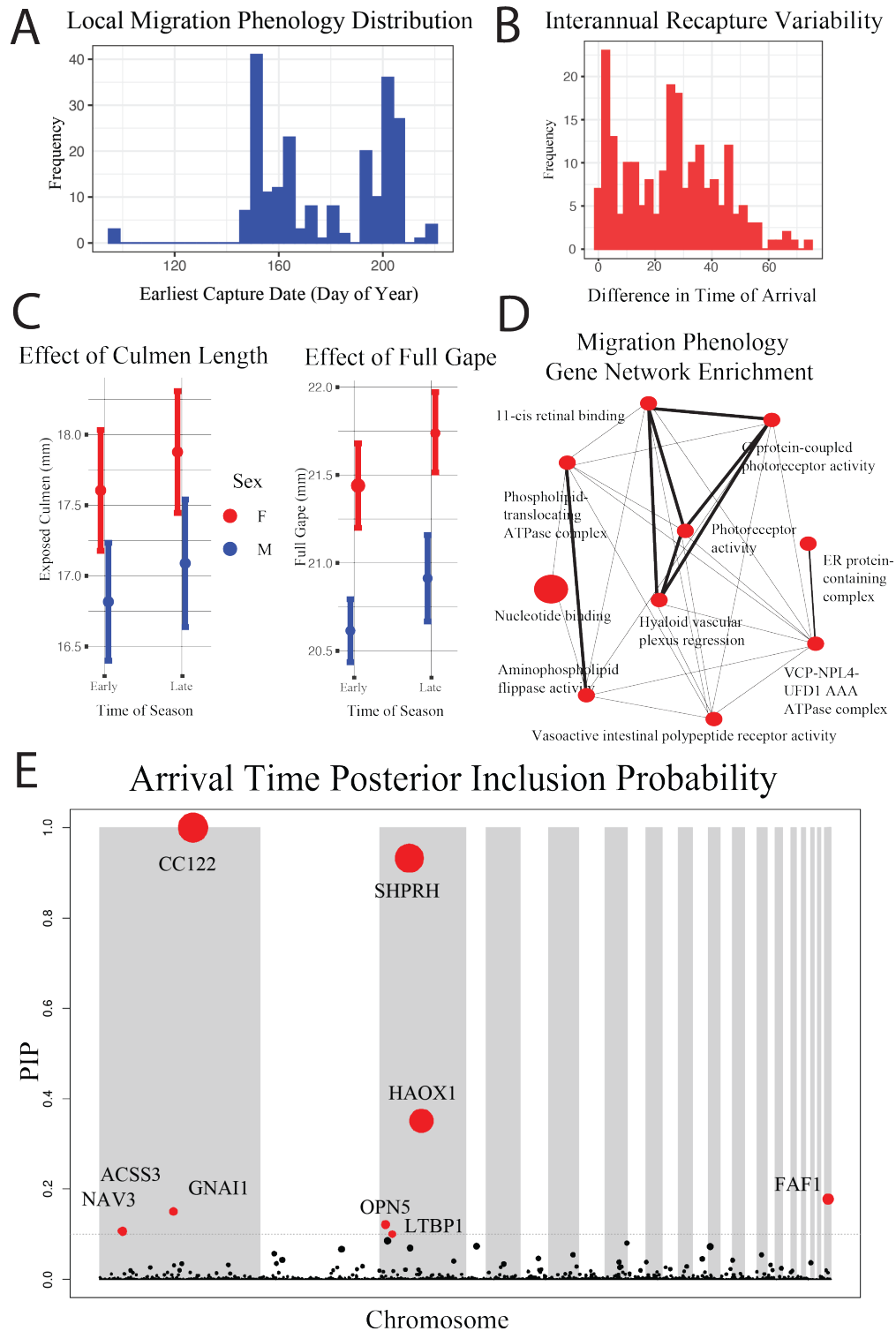


Figure 5. Variation in migration phenology is largely heritable and linked to variation in bill length. A. Local migration phenology is bimodal, with a wave of individuals arriving in the early summer, and a subsequent wave of local migration in the late summer. B. Birds are recaptured consistently in the early vs. late summer between years, with the majority of earliest local recaptures occurring in the same month they were first captured in previous years. C. Both measures of bill length are longer in the late versus early summer, overlapping with early and late waves of local migration. Plots represent marginal effects of linear mixed models (Full gape p-value= 0.02684, Exposed culmen p-value= 0.02485). D. Migration phenology gene network showing enriched terms for local migration phenotypes. Terms were enriched for photoreceptor activity and hyaloid vascular plexus regression, which is involved in development of the eye. E. Posterior inclusion probability plot revealing nine candidate loci with large effects on migration phenology as measured by BSLMM. Genes had functions related to migration in birds including circadian rhythm, fatty acid metabolism, oxidative stress, and aggressive behavior.

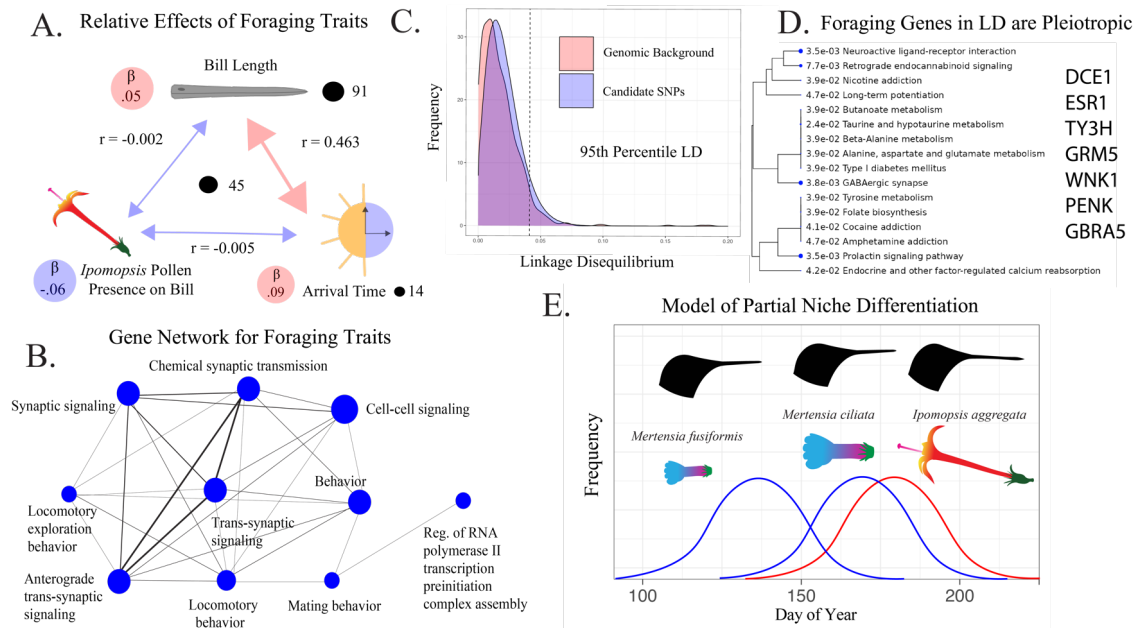


Figure 6. Genomic architecture of foraging traits is interactive. A. Bill length and arrival time are genetically correlated for large effect loci with weak association to *Ipomopsis aggregata* pollen prevalence. β denotes the average effect size of shared SNPs on each phenotype; r is the genetic correlation coefficient. Black circles refer to the number of SNPs in each group. B. Gene networks for foraging traits are enriched for locomotory exploration behavior. C. Pairwise LD was the same for candidate SNPs compared to the genomic background, but had a higher variance and a right skewed tail. D. SNPs were identified above the 95th percentile for pairwise LD in candidate SNPs. A subset of these had long range LD between chromosomes with highly pleiotropic effects involved in behavior and morphology, many of which were jointly involved in calcium ion homeostasis and learning. This suggests that SNPs linked to seasonality of migration and bill length are co-selected across the genome. Enrichments for genes with known pleiotropic effects were enriched for calcium ion reabsorption, addictive behavior, and most significantly with prolactin signaling, which regulates seasonality. E. Model of partial niche differentiation based on phenology of flowering, migration, bill size, and pollen prevalence. Birds with shorter bills in the early season have higher probability of carrying *Mertensia* spp. pollen, which produce flowers with shorter nectar tubes. The arrival of longer-billed birds in the late summer revealed partial niche partitioning between short and long flowers by bill phenotypes.

Supplemental Methods

Whole Genome Resequencing

All sampling of BTH was conducted from 2015 to 2019 in Gunnison County, Colorado at 16 sites listed in Supplementary Table 1 using methods from (40). Birds were live-captured and released after these procedures in accordance with ACUC, state, and federal regulations. A single drop of blood ≤ 10 microliters was collected from each bird and mixed with Longmire's buffer in a 2 mL cryovial for downstream whole genome resequencing (68). Approximately 300 microliters of buffer mixture were extracted from each individual cryovial and placed in a lysis buffer for 4-8 hours. Each sample was then extracted for DNA using a modified phenol-chloroform extraction followed by bead purification. Total DNA was resuspended in distilled water and assessed for quality using the broad range Qubit, agarose gel electrophoresis, and Bioanalyzer readings. Three different library preparation kits were used on different subsets of birds for comparison: Nextera DNA Flex, Kapa Hyperprep, and Seqwell Low Input LP 384. The majority of libraries were prepared with the Seqwell Low Input kit. All samples were then sequenced on the HiSeq 4000 or the NovaSeq 6000 to an average whole genome coverage of 3x across samples (Supplemental Table 2).

Adjusting Phenotypes for Covariates

Phenotypes are often correlated and can generate misleading associations in GWAS. Bill size in birds, for example, is generally positively correlated with body size (69) and leads to spurious associations between genotype and bill size (43). To correct for body size, we first identified phenotypes that covary with each trait of interest using multivariate linear models and subsequently curated the residual phenotypes for downstream GWAS to prevent such spurious associations. Residual phenotype data were generated in R using linear mixed models between each phenotype and a panel of fixed or random effects that could be relevant based on the literature. We then generated a qqplot as a first pass to determine if any variables needed to be dropped. This was followed by a backwards stepwise elimination of nonsignificant effects using the step function from the lme4 package (70). A follow-up quantile comparison plot was produced to determine if residuals were from the same distribution, and if so, the residuals for that phenotype were extracted. We were unable to generate appropriate quantile plots for a subset of phenotypes, so some response variables were transformed. We used the bestNormalize package in R to determine the most appropriate transformation of each response variable (44). The same model selection process and extraction of residuals was done following these transformations.

Most phenotypes were associated with sex, for which we take the residuals of said phenotypes. Sex differences are expected in most traits for this species as the 'Mellisugini' tribe of hummingbirds, which includes the BTH, is sexually dimorphic for size-related traits (71). We did not control for age as all samples were collected until mid-July and most fledging occurs in August (72). Residual phenotypes for full gape, exposed culmen, and mandible width controlled for wing chord, as a proxy for body size, and sex. Bill depth, in contrast, only included sex as a covariate. Bill width required a log transformation, but also included the same covariates as bill length measures. Curvature required a boxcox transformation and included wing chord and exposed culmen as covariates. We tested whether exposed culmen covaried with curvature because longer bills are occasionally more decurved in hummingbirds and curvature was subsequently included as a covariate in the generation of residuals (73).

We created a new variables representative of shape using existing phenotypes as phenotypes for downstream GWAS as a ratio between bill dimensions is suggestive of shape rather than size. These included the ratio between culmen length/width and culmen depth/length. We generated residuals for culmen length/width using wing chord and sex as covariates following a log transformation for normalization. Wing chord included only sex as a covariate and had two outlier points removed. The first two principal components of dorsal and profile shape only included sex as a covariate. Dorsal shape PC1 required the removal of two outliers and a yeojohnson transformation. Dorsal shape PC2 only required the removal of three outlier points. We removed two outlier points and administer a square root transformation of profile shape PC1, and apply an ordered quantile normalization transformation to PC2. Finally, we created a variable for migration phenology, day of year (DoY), representing the day of year birds were first captured, and applied an ordered quantile normalization transformation, including sex as a covariate.

Resequencing Read Quality Assessment

We began the processing of paired-end short read data using Grenepipe, a snakemake (5.19.3) based pipeline, which uses a suite of software to provide quality control metrics and generate sorted binary alignment maps (bam) (74). Adapters were trimmed using fastp (0.64.0) with merge-paired-end-reads set to false (75). Sequence quality and adapter content were assessed using fastqc (0.11.9) and summarized with multiqc (1.10.1) before and after running the adapter trimming step to confirm adapters were removed (76, 77). Trimmed, paired-end reads were then aligned to the genome using bwa mem (0.7.17) without filtering mapped reads, then sorted and indexed with samtools v1.12 (78, 79). PCR Duplicates were then marked and sorted with Picard v.2.23.0, then sorted and indexed again with samtools (80). We then calculated the sequencing depth of each alignment map before and after PCR duplicate removal using samtools (1.8) depth to determine if any samples should have been excluded due to poor library quality.

A multiqc summary of the 510 binary alignment map files show that <10% of data across all files were removed as duplicates. The entire sample had an average coverage of 3.45x and ranged between 1.06-14.56x coverage, where deduplication reduced the average coverage to 3.12x (Supplementary Figure 8) where the mean loss of coverage was 9.75% of the unfiltered alignments. The lowest average sequence length across samples was 106 bp, although the majority of samples were between 135-150 bp. Samples ranged from 0.02- 93.44 million reads with the majority ranging from 3-32 million reads. All samples had a phred score above 30, ~0 per base N content, <1% adapter content, and varied between 40-44% GC content.

Assessment of the reference genome in the context of read alignment was performed using linear models of scaffold size against the mean and standard deviation of base quality, map quality, and coverage across all sites. We identified 23 scaffolds as outliers for at least one of these metrics (Supplementary Figure 9). While there were four outlier chromosomes for mean base quality, the range of mean base qualities was small, so were not considered scaffolds of interest. Chromosome 32 was identified as being the only outlier chromosome for mean coverage, mean map quality, and the standard deviation of coverage having unusually high coverage so was excluded from autosomes for some downstream analyses. Three outlier chromosomes were identified each for the standard deviation of base quality and map quality, but the range across values were small so were not considered scaffolds of interest.

Scaffold Quality Assessment

Genome-wide coverage was calculated for each individual using samtools (1.6). All BTH were above 2 standard deviations below mean genome-wide coverage (3.12X) across all individuals, so no individuals were excluded from our dataset. We then used samtools (1.10) to calculate coverage for each scaffold in all individuals to determine if any scaffolds were outliers for map quality, base quality, or coverage (81). These data were imported into excel to calculate a mean and standard deviation for each metric in each scaffold. Data were imported into R along with chromosome sizes for each scaffold (82). We ran a series of linear models of the scaffold size against the mean or standard deviation of coverage, base quality, or map quality. Chromosome 32 was consistently an outlier and was split into 200Kbp fragments using Samtools 1.8. We wanted to determine if this scaffold shared similarity with the W chromosome, which could produce mismapping from the female samples. We tested this using a blastn search in the NCBI database with these sequences and were determined to have high similarity to the *Calypte anna* and *Caprimulgus europaeus* W chromosome or *Streptopelia turtur* Z chromosome. We concluded that this abnormal coverage was due to pseudohomologous sequence shared between this autosome and the sex chromosomes. The Z chromosome was identified using a blastn (2.6.0) search using the Chicken (NC_052572.1) Z chromosome as a query, identifying chromosome 4 in our assembly (40, 83). We then assessed coverage of chromosome 4 for all birds using Qualimap to confirm sex (2.2.2)(84).

Total Site Coverage Filter

Working with low coverage whole genome data requires a filtering process that excludes sites with unusually high coverage (repeat content, contamination, misassembly) and unusually low coverage (sequencing errors). This could be achieved by plotting a histogram for counts of variable sites. We were only interested in the distribution within around two standard deviations of the mean, excluding sites outside of this region. The component of this plot depicting exponential decay on the left of this bell curve primarily consisted of sequencing errors, while coverage far outside the right tail of the bell curve were likely repeat content mismappings or contamination. The assignment of cutoff values was aimed at removing most of these sites from consideration. We generated three separate plots for chromosome Z, chromosome 32, and autosomes excluding Z/Chr32 as these were expected to have different distributions of SNP count frequencies. We first generated a list of variable sites with a minimum mapping quality of 30 and a maximum depth of 10000 then created a table of counts for the number of sites that have a sequencing depth of x across all individuals using ANGSD 0.935-33-g79d9455 (htslib: 1.12-36-gbf32d56) (85). The tails of the bell curve were then used to assign the minimum and maximum depth of each variable site across all samples.

Mappability Filter

We wanted to consider only sites in the genome that were mappable in order to reduce memory and time requirements for downstream analyses. The unique alignment of kmers to the reference genome would then allow us to increase the probability of reads mapping uniquely. We use the program Genmap to compute the uniqueness of kmers for each position in the genome (86). Kmers of size 50 were realigned to the genome and the percentage of the genome represented by non-unique mappings was computed. We wanted to determine an appropriate cutoff based on the proportion of sites being masked and retained only sites that mapped uniquely to one site. This effectively reduced our genome coverage by ~5%.

Per Site Filters

Next, we generated a list of positions that passed a panel of filters for downstream analyses. The first step of this process was generating a summary of coverage for each sample using bcftools (1.13) mpileup (87). We set a maximum depth and indel depth of 100 million and a minimum base quality of 0 for this step. This allowed us to generate a consensus for each site using bcftools call. We then filtered out only the unmappable portion of the genome from the previous step and excluded indels, sites with a map quality less than 30, and sites above 20,000x coverage. We then selected for sites with biallelic SNPs and filtered for strand bias, base quality bias, map quality bias, and tail distance bias if p-values were below $1e-100$. We also filtered out sites with deviations from Hardy-Weinberg equilibrium if p-values fell below $1e-6$. We labelled sites where alleles were missing because of an overlapping variant, then implemented a custom perl script to remove various types of multiallelic sites.

Minimum site filters included a map quality of 30 and a maximum site depth per sample of 10K using ANGSD. A histogram of coverage per site across all samples was generated for Autosomes, the Z chromosome, and the outlier Chromosome 32 from the previous step (Supplementary Figure 10). The minimum depth per site across all samples was set to 580 for Autosomes and 500 for the Z chromosome based on the distributions of coverage. Due to the lack of normally distributed coverage for Chromosome 32, the minimum depth per site was set to 580 along with other Autosomes. We then further restricted sites to only mappable regions of the genome using Genmap (86). Sites were restricted to regions where 50mers aligned uniquely, which reduced the mappable portion of the genome to 967655545 base pairs, or 95.8% of the original genome size. Following additional bcftools filters for biallelic SNPs, strand bias, base quality bias, map quality bias, tail distance bias, and deviations from Hardy-Weinberg equilibrium, a further 2 Mb of sites were removed and >95% of the genome was still retained for downstream analysis.

The abnormal coverage and map quality distribution of Chromosome 32 along with the similarities to Chromosome W from *Calypte anna* suggest that pseudohomologous sequence to the W chromosome exist on this chromosome. Because our reference genome is male, there are no true W chromosome sequences to align to, but W chromosome sequences in female samples may misalign to this region. We used nsgParalog to identify sites problematic for short read mapping that relies on abnormal coverage or heterozygosity to supplement this issue across the genome. Roughly 32399982 SNPs were identified in ANGSD using the aforementioned filters along with additional filters: base quality of 20, SNP call with pvalue $<1e-6$, MAF of 1%, at least one read present per site in $\geq 60\%$ of the samples, and a genotype posterior probability $\geq 95\%$. Following ngsParalog, 31857673 SNPs did not show evidence of mismapping, with a retention of 98.2% of identified SNPs. SNP calls using these filters in ANGSD with an updated site list produced 31856133 SNPs above 1% MAF, 2069282 above 5%, and 4693 above 10%.

Accounting for Mapping to Pseudohomologous Regions

Results from the previous step provided evidence for pseudohomology between autosomes and sex chromosomes W and Z. The failure to exclude positions that mismapped in these regions would result in genetic structure between males and females as the W chromosome is only present in females. We began to deal with this artifact by generating a large list of genotype likelihoods for biallelic sites with a liberal filter in ANGSD (85). Minimum minor allele frequency was set to 0.01, minimum map quality set to 30, minimum base quality set to 20, maximum depth for each site across all individuals was set to 2300, and a liberal p-value cutoff was set for SNP calling at $1e-6$.

We also estimated the posterior genotype probability of each site and included only sites with a posterior probability above 95%. We required that the minimum depth in each site was at least 1x in 306 individuals (60% of total sample). An additional filter for a minimum depth per site of 580 for autosomes and scaffolds, and 500 for the Z chromosome (Chr4), were implemented based on the total site coverage step. The sites generated from this step were constrained to overlap only sites passing previous filtering steps.

Coverage data were generated across these sites using samtools mpileup with maximum depth set arbitrarily high at 3756000 (81). The pileup output was then input into ngsParalog which detects genomic regions problematic for short reading mapping. The output of ngsParalog is a likelihood estimate for the probability that reads covering a particular site are mismapped based on genotype likelihoods (88). We then filtered out sites below a bonferroni-corrected p-value for mismapping.

Principal Component Analysis

Principal component analysis is commonly used as a means of identifying structure in genotype/genotype likelihood data. Structure in the genotype data can often occur through population structure, relatedness among individuals, missing data, sex-specific mapping, or other artifacts of the preparation process such as library preparation kit, sequencer, or batch of samples. We used an updated positions file from the NgsParalog output and applied the same filters above in ANGSD 0.937-92-g5cd355b (htslib: 1.15.1-35-gf46597e) and produced a beagle likelihood file. The beagle file was then input to PCANGSD (0.99) with three different minor allele frequency (MAF) filters: 0.01, 0.05, and 0.10 (89). The output covariance matrices were then imported into R and principal components were plotted using the prcomp and autoplot functions from the ggfortify package (82, 90). We colored variables such as sex, library preparation kit, sequencer, or coverage to infer structure in the data.

Relatedness Estimation

The percent of individuals recaptured between growing seasons for BTH between breeding seasons can be as high as 70% for females and 27% for males (50). This creates the risk of structured data in the form of cryptic relatedness if there is philopatry among relatives. We empirically estimated pairwise relatedness between all individuals in our dataset using NgsRelate (91). The number of comparisons run by NgsRelate is memory-intensive, so we subset the filtered positions file every 50 sites. We then ran the same filters from the previous step in ANGSD to generate an output file in GLF instead of Beagle format. The GLF file was then subset to include only autosomes, excluding Chr32, unplaced scaffolds, and the Z chromosome. NgsRelate was then run with this genotype likelihood file along with a list of SNP positions and their respective minor and major allele frequencies. We then plotted the frequency of all pairwise estimates of relatedness as a scatterplot using the base R plot function (82).

We wanted to exclude the possibility that birds with similar DoY values are related, resulting in similar phenotypic values. This could create a false signal of average trait shifts over time. All pairwise relatedness values were first included in a distance matrix and all pairs that were sampled in different sites were set to NA. We then created a separate distance matrix for the absolute difference in the average earliest DoY for each growing season between individuals. If birds sampled around the same time are more related to each other than two birds sampled randomly, then smaller differences in day of year should positively correlate with degree of relatedness. Regressing difference in the day of capture against pairwise relatedness values

would violate the assumptions of classic linear regression as values are not independent. We instead calculated a Mantel statistic which tests for the correlation between dissimilarity matrices using the package ‘vegan’ in R (92). Because this does not directly calculate a p-value, we use 1000 permutation tests and calculate a p-value for the Pearson correlation between day of year and pairwise relatedness.

Site Frequency Spectra Estimation

Many population genetic inferences can be made from the site frequency spectrum (SFS), which summarizes the distribution of allele frequencies in a population. We estimated the SFS in ANGSD from autosomes, excluding chromosome 4, chromosome 32, and unplaced scaffolds. We generated a sample allele frequency (SAF) file, containing likelihoods of sampling k-derived alleles, using the same filters as the principal component analysis with two exceptions. We used the positions file before the ngsParalog filter was applied as it contained alleles with a MAF below 1%. We additionally increased the p-value threshold to 1e-4 instead of 1e-6 as the latter was too conservative for identifying low MAF alleles. We then estimated the folded SFS in ANGSD using the output SAF file.

Inbreeding within populations can affect the shape of an SFS due to excess homozygosity, which creates a characteristic hill in mid- to high-frequency bins (93). This is a concern for populations in which philopatry is consistent over many generations (94). We estimated the inbreeding coefficient in each individual using NgsF to control for this potential effect (95). We used the same GLF file from the NgsRelate step consisting of 622,255 autosomal sites and a minimum allele frequency of 1%. The inbreeding coefficients output from this step were then included in the generation of a new SAF file using the same filters as the previous SFS generation step. This permitted the generation of SFS based on per site posterior probabilities controlling for inbreeding coefficients or assuming HWE in the previous step. distribution of inbreeding coefficients were visualized with ggplot2 in R.

Genotype Imputation and Validation

The imputation of genotypes or genotype likelihoods requires an estimate of the coalescent effective population size (N_e), a parameter used in the calculation for HMM transition probabilities between two markers (96). We used the folded SFS from the previous step, not controlling for inbreeding, in order to estimate this. Theta was estimated at every site and summarized for each chromosome in ANGSD. We solved for N_e by dividing the pairwise theta by the mutation rate scaled by chromosome size. The mutation rate used for this calculation was the germline mutation rate for birds ($4 \times 4.6 \times 10^{-9}$) from (97). The average of all autosomal estimates (excluding Chr32, ChrZ, Unplaced Scaffolds) was 63012. Imputation error rates are typically only affected when diversity is less than human populations ($N_e=10,000$) (98), and are insensitive to parameter values between 10^4 - 10^6 (99).

Imputation was performed using BEAGLE 4.1, which accepts genotype likelihoods as input (100). A large combined VCF file for all filtered sites was generated using output BCF files in ANGSD with the same filters from the PCA step. BCF files were first converted into VCF format with bcftools view (1.13) and then merged with bcftools concat (87). Each scaffold was then divided into 10Mb nonoverlapping intervals with bedtools makewindows (2.28.0) and imputed in BEAGLE with the N_e parameter set to 64000.

Imputation accuracy was assessed by first randomly selecting 1% of sites from the unimputed VCF for all SNPs. We then set genotypes for samples with greater than or equal to a

genotype probability of 0.999 to missing and then imputed with BEAGLE 4.1 using the same parameters. A concordance table was generated with bcftools stats between the imputed subset file and the original subset file to summarize discrepancies in predicted genotypes (101). We then report the reference homozygote accuracy, heterozygote accuracy, alternative homozygote accuracy, and r^2 as a function of allele frequency with plot-vcfstats (87).

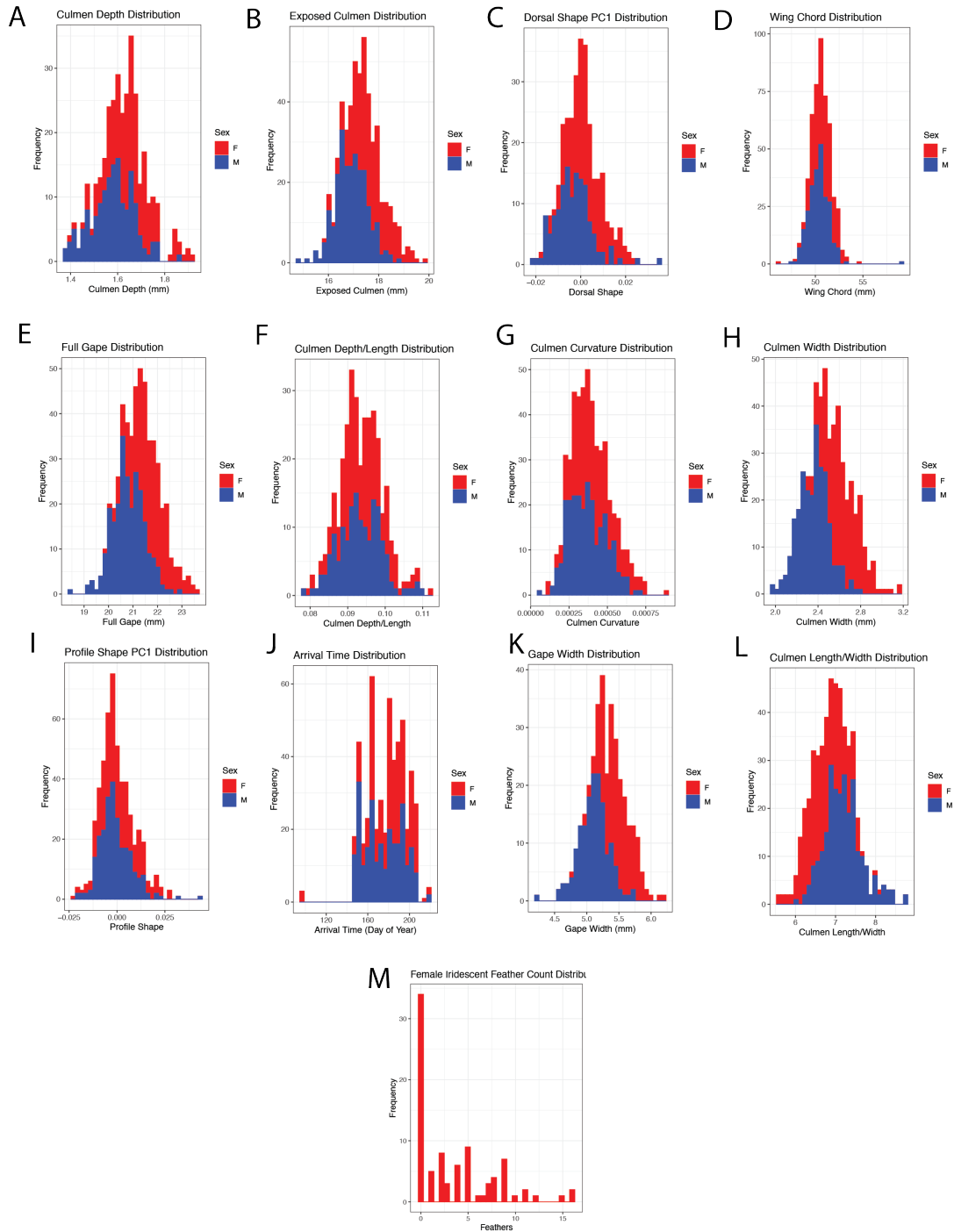
Our data were low coverage with a mean sequencing depth of 3x per sample, so missingness was expected to be high. SNP filters for this dataset allowed for a minimum of 1x coverage per site in 60% of the individuals, which consequently allowed for up to 40% missingness. We compared imputed data and data with simulated missingness + imputation to validate imputation accuracy. Accuracy was measured by the squared correlation between imputed and true genotype as well as the concordance between predicted and true genotypes, both as a function of allele frequency. Imputation accuracy measured by R^2 was >99% accuracy for allele frequencies greater than .003%. Reference homozygotes and heterozygotes had a concordance rate of >99% for non-reference allele frequencies of at least 1%. Non-reference homozygote concordance was slightly lower with a concordance rate of 97% for allele frequencies of at least 1%, reaching 99% when allele frequency was at least 3% frequency. All concordance rates for non-reference allele frequencies of at least 1% were based on 27,654,828 genotypes. Of this set, 24200795 SNPs had a MAF of at least 1%, 1344060 with at least 5%, and 3410 with at least 10%.

LD Pruning

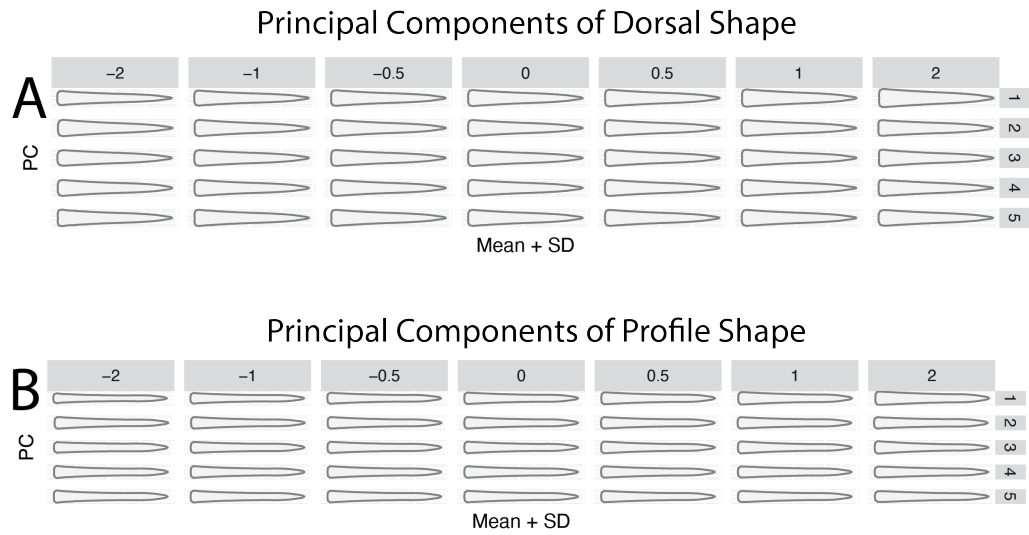
Significance thresholds for GWAS are overly conservative when association tests between SNPs are nonindependent due to linkage disequilibrium (LD) (102). SNPs identified in GWAS are also commonly used in estimates of heritability, which can be overestimated when linkage is unaccounted for in effect size estimation (38). We used the genotype likelihood files in beagle format from previous steps to calculate LD between variants on each scaffold. The number of LD calculations are a function of the number of variant sites per scaffold, which can be computationally intensive when the number of pairwise comparisons are high.

We first determined the average size of linkage blocks to reduce the number of pairwise comparisons by plotting LD decay for the largest chromosome. This was visualized using the Lddecay function in the NgsLD package (103). The number of comparisons can still remain high when a scaffold has many variants, particularly on large chromosomes, even when LD blocks are relatively small. We additionally subsampled the number of comparisons as a function of scaffold size: .01% for Chromosome 1-14, .1% for chromosomes 15-18, and 1% for chromosomes 19-33 and unplaced scaffolds. This allowed us to generate a list of LD-pruned positions with a maximum kb distance of 20kb between variants and a minimum LD estimate of 0.1. The final number of SNP positions were reduced from 31856132 to 31629590 by around 226,000 SNPs. The modest reduction in SNPs after pruning resulted from a relatively large number of low frequency SNPs which contributed little to linkage. This could also be seen in a plot of the decay in R^2 across chromosome 1 showing an average block size of 10 kilobases (Supplemental Figure 15).

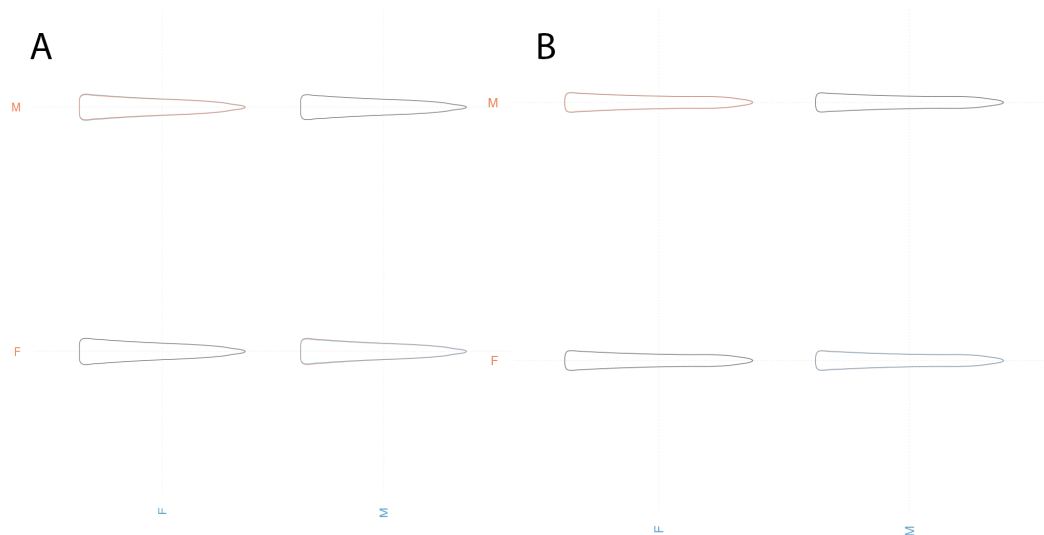
Supplementary Figures



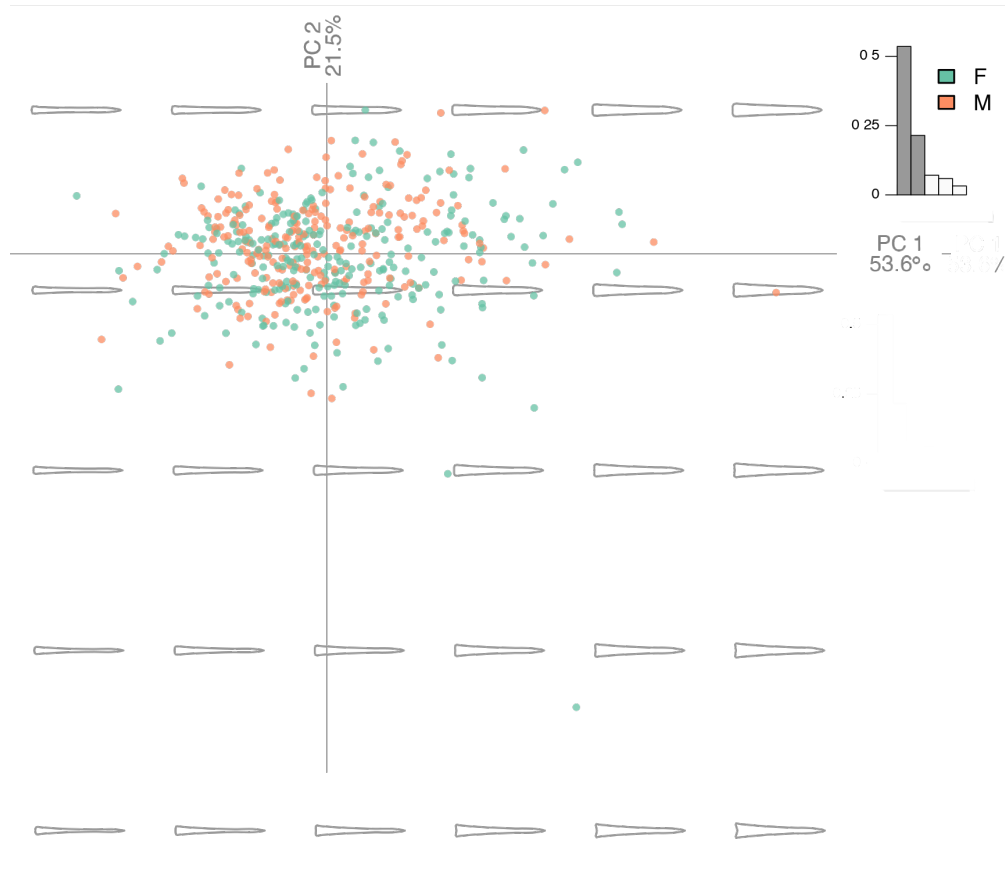
Supplementary Figure 1. Distribution of phenotypes related to bill dimensions by sex along with summary statistics. A. Culmen depth. B. Exposed culmen. C. Dorsal shape PC1. D. Wing chord. E. Full gape. F. Culmen depth/length ratio. G. Culmen curvature. H. Culmen width. I. Profile shape PC1. J. Arrival time. K. Gape width. L. Culmen length/width ratio. M. Iridescent feather count in adult females.



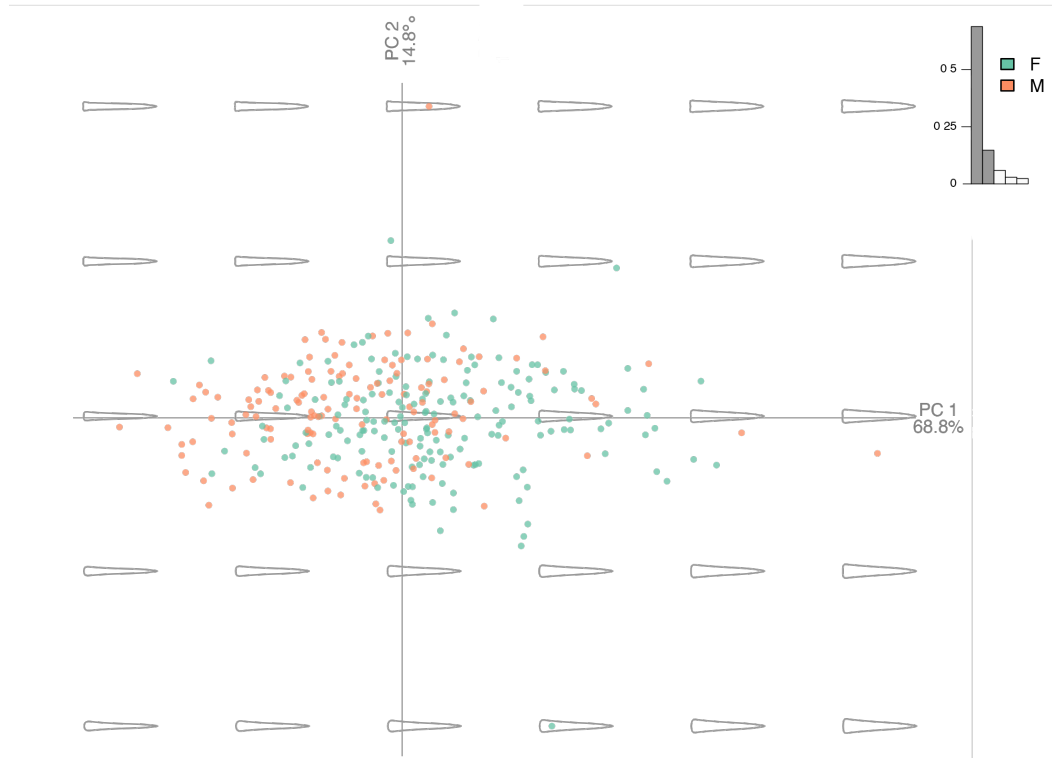
Supplementary Figure 2. A. Principal components of dorsal shape and (B) profile shape. Each row depicts principal components 1-5 for each phenotype while columns indicate the standard deviations in bill shape for each principal component. Note: profile shape included the outlines of the maxilla and mandible.



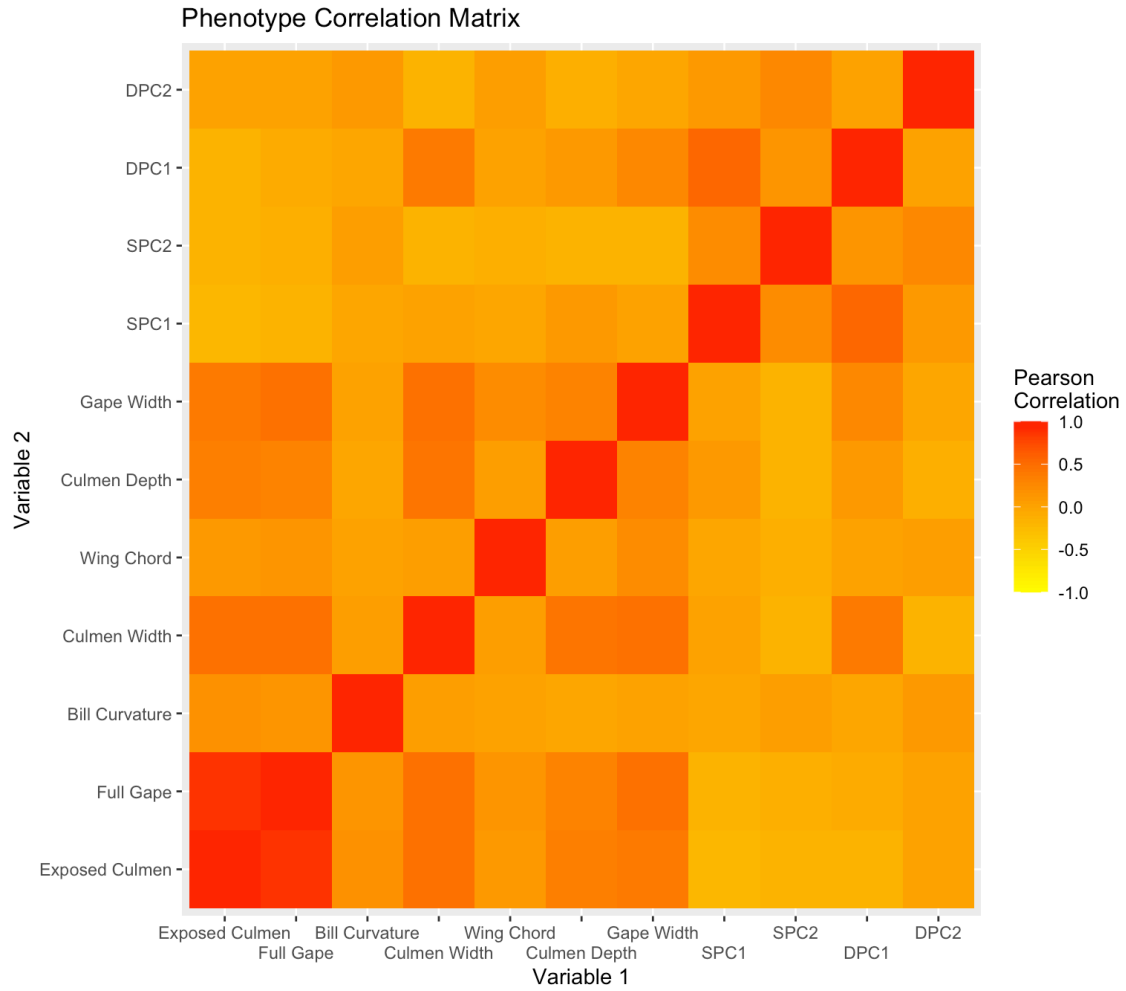
Supplementary Figure 3. The average dorsal shape (A) and profile shape (B) of males and females. The bottom left of each panel depicts the average shape for a female with male shapes on the top right. Each off diagonal shape shows the overlap between the two conditions to highlight subtle differences. Male and female bills, although they differ in size, do not differ in shape on average.



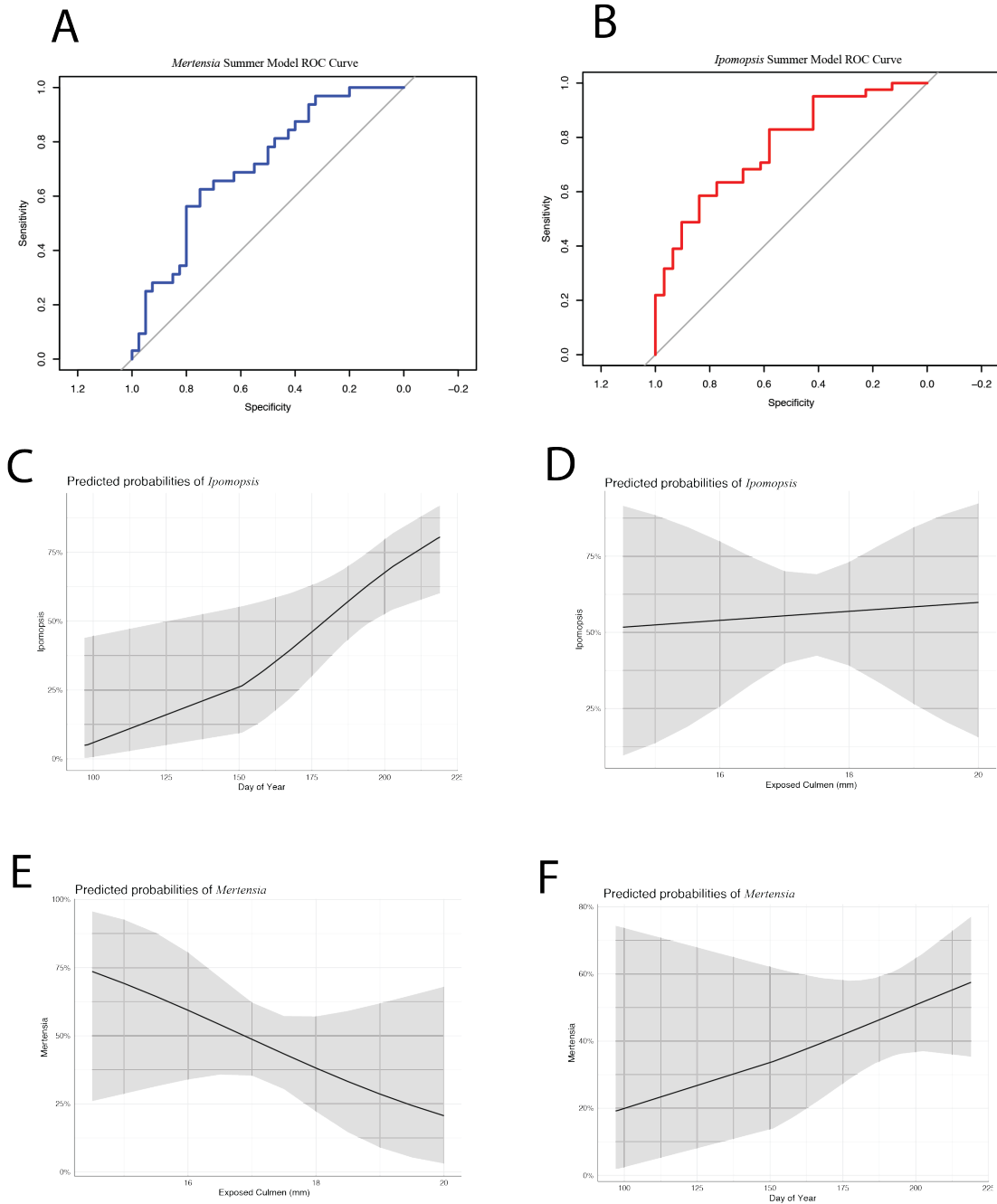
Supplementary Figure 4. Distribution of samples in shape space plotted along principal components 1 and 2 for profile shape. Principal components 1 and 2 represented 53.6% and 21.5% of variation in profile shape respectively.



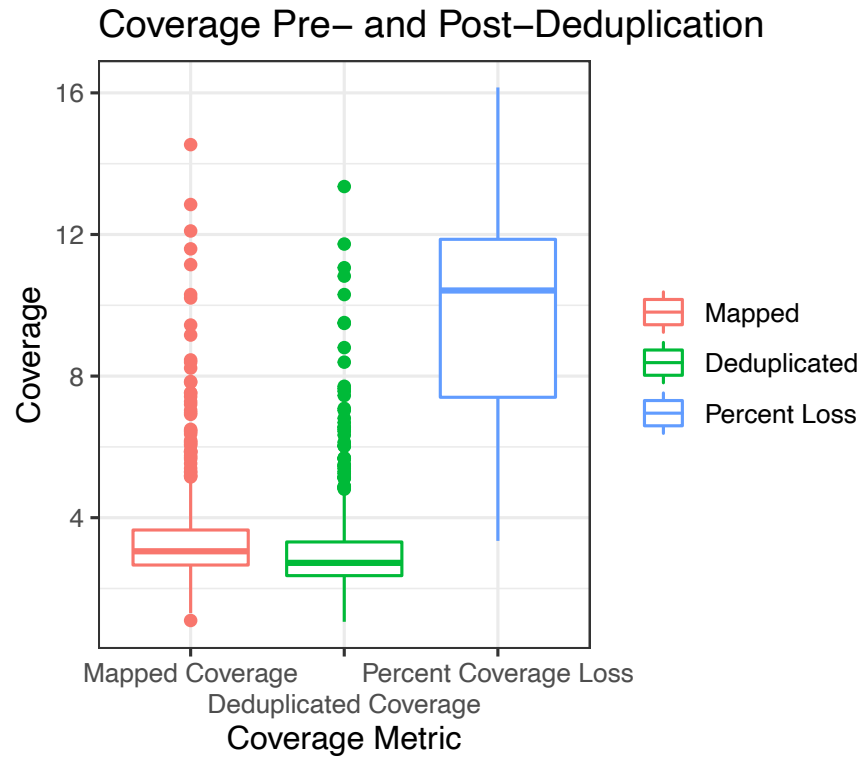
Supplementary Figure 5. Distribution of samples in shape space plotted along principal components 1 and 2 for dorsal shape. Principal components 1 and 2 represented 68.8% and 14.8% of variation in dorsal shape respectively.



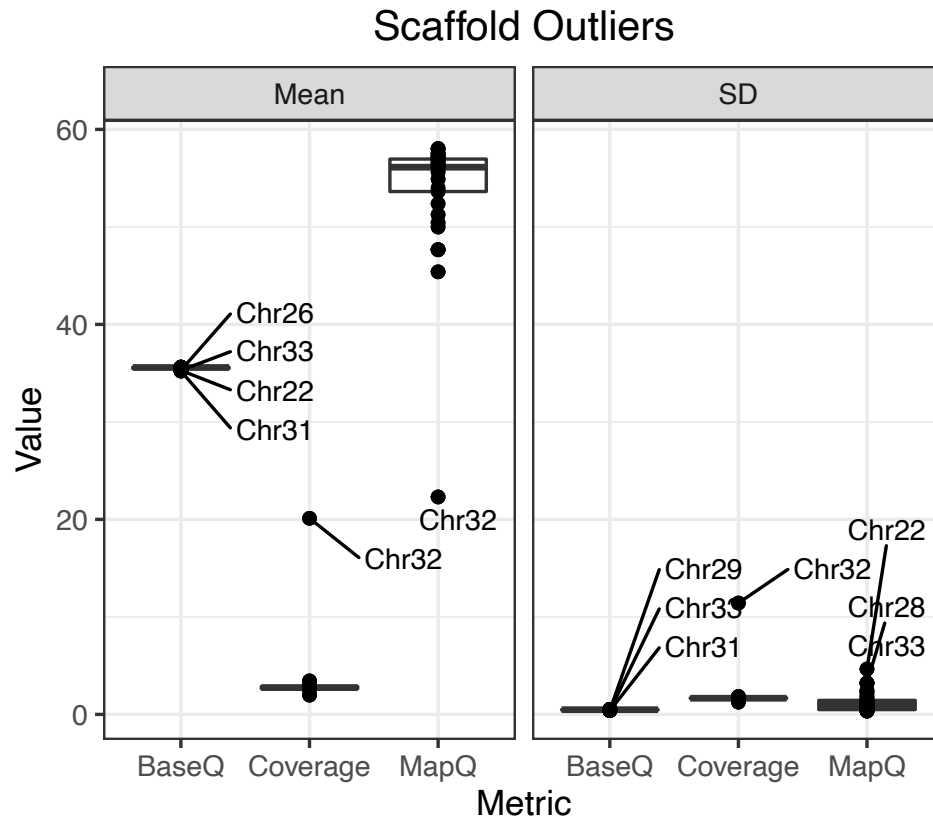
Supplementary Figure 6. Heat map depicting the Pearson correlation between phenotypes. Trait pairs with yellow or red are indicative of smaller and larger values of the correlation coefficient ‘ r ’, respectively.



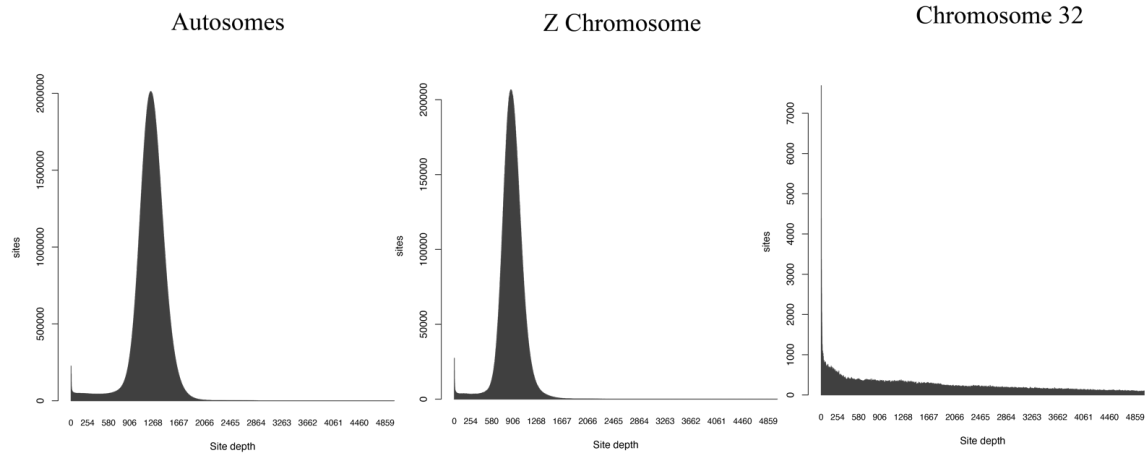
Supplementary Figure 2. Prediction metrics for generalized linear models of pollen presence/absence. Panels A and B show the true positive rate (specificity) plot against the false positive rate (sensitivity) as a metric for performance of the GLM at a different classification thresholds. A. ROC curve for the *Mertensia* summer model. B. ROC curve for the *Ipomopsis* summer model. Figures C-F depict the estimated marginal means of exposed culmen and DoY for both summer models of *Ipomopsis* and *Mertensia* pollen prevalence.



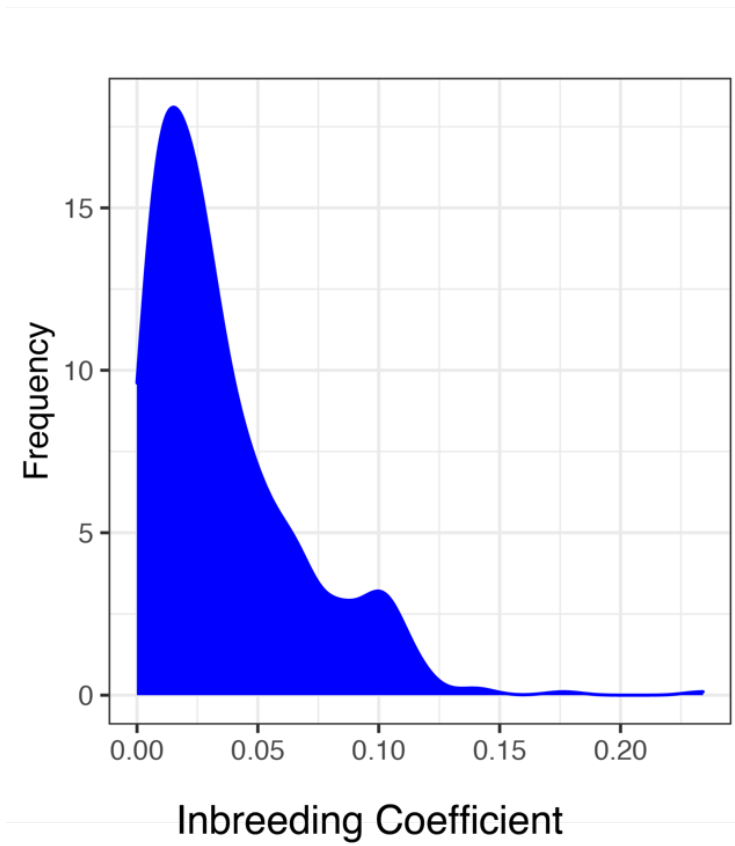
Supplementary Figure 8. Boxplots depicting the coverage of mapped reads in binary alignment map files before and after PCR duplicate removal with Picard Tools. Also show in the distribution of coverage loss.



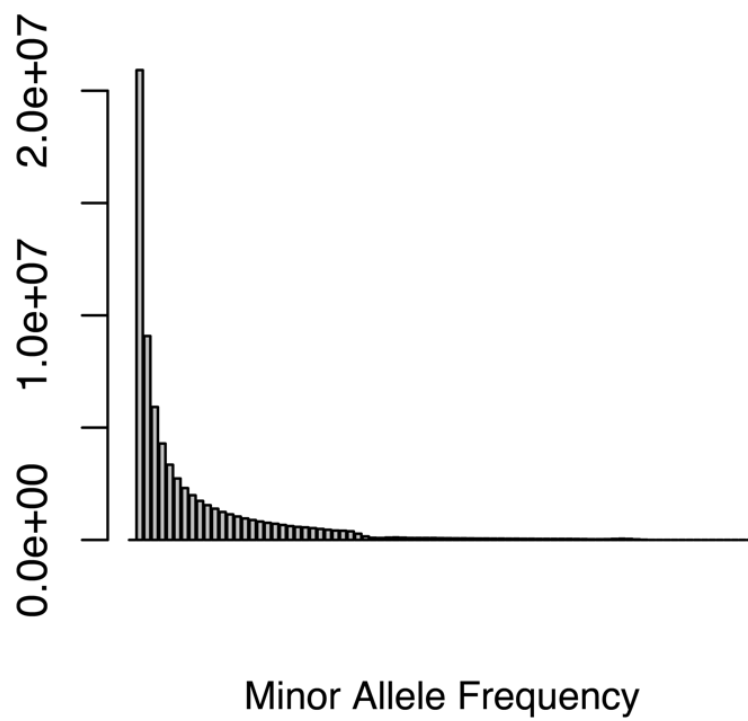
Supplementary Figure 9. Outliers for linear models of scaffold size against various summary metrics for all sites. Listed are chromosomes that were identified as outliers for the mean value or standard deviation of base quality, map quality, or coverage.



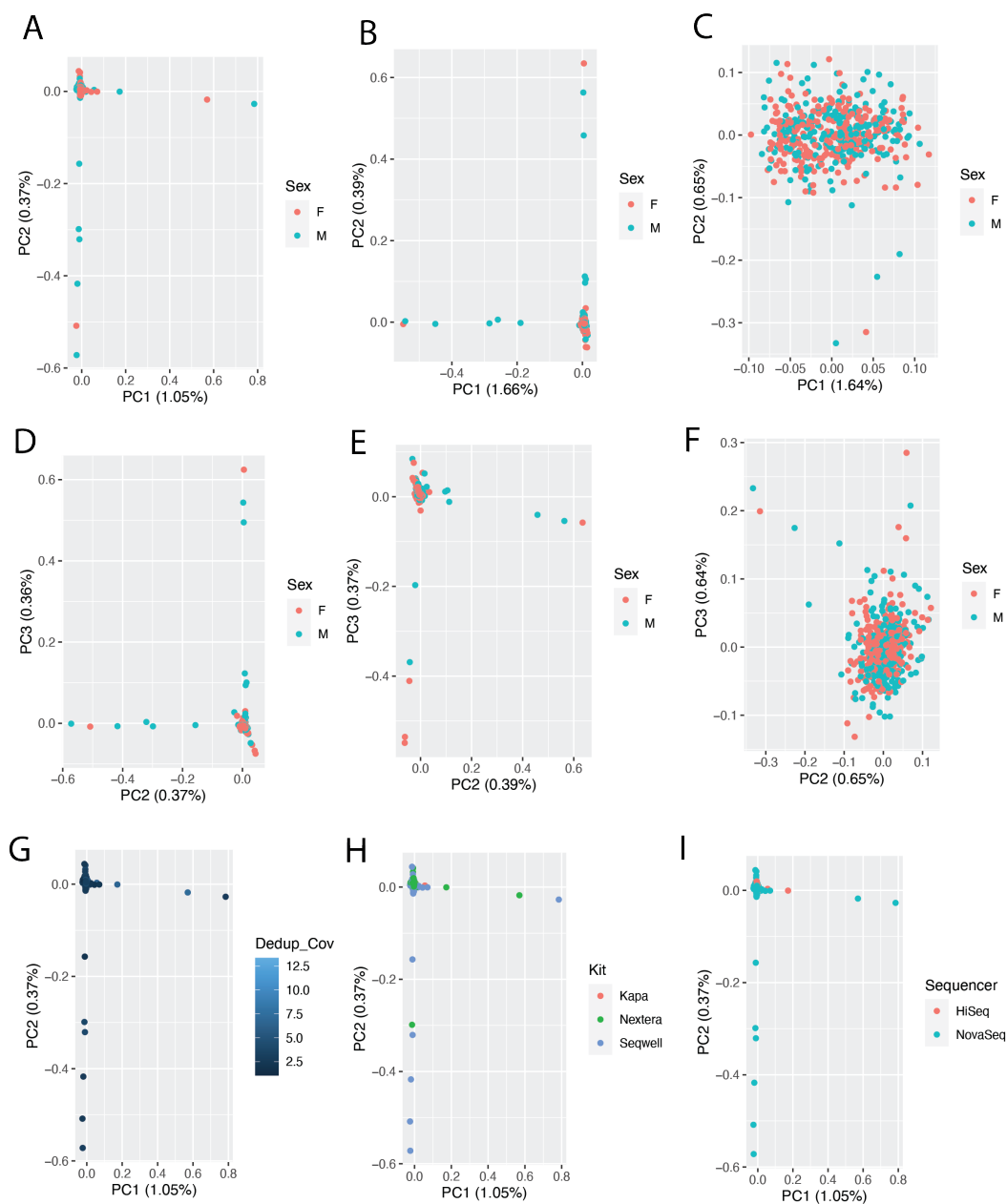
Supplementary Figure 10. Histograms of coverage per site across all samples. Autosomes in the left panel excluded data from Chromosome 32 or unplaced scaffolds. The low coverage cutoff was set to 580x for Autosomes, 500x for the Z chromosome (middle), and was set to Autosome cutoff for Chromosome 32. The maximum depth cutoff was set to 2300 for all three chromosome types.



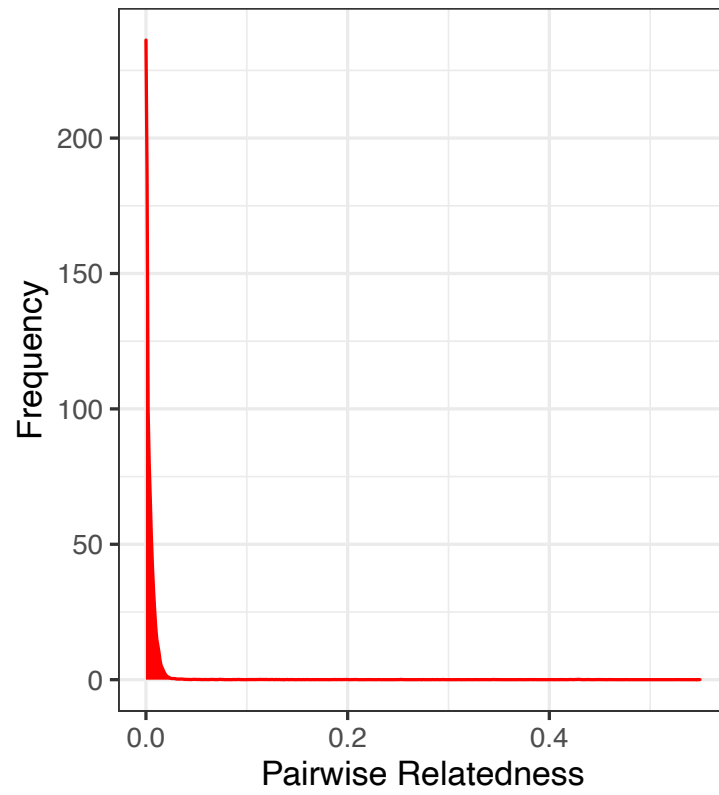
Supplementary Figure 11. Distribution of inbreeding coefficients for all samples. Inbreeding coefficient distribution was low enough such that the site frequency spectra did not require estimation of genotype probabilities conditioning on inbreeding coefficients.



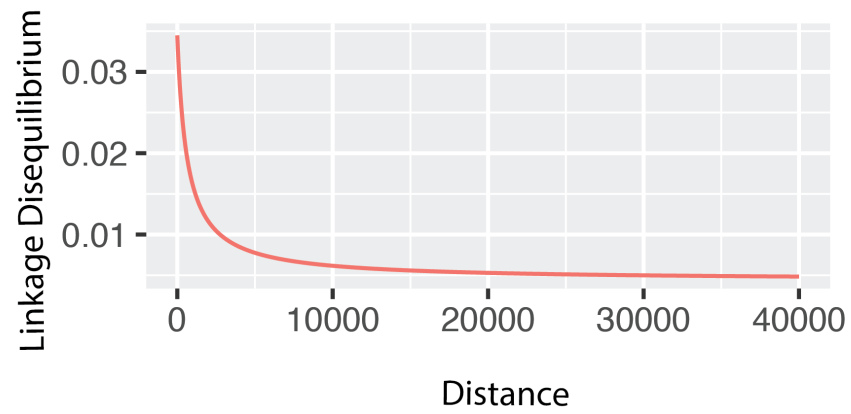
Supplementary Figure 12. Standard folded site frequency spectrum.



Supplemental Figure 13. Principal components of genotype likelihoods with a minimum MAF threshold of A. 1%, B. 5%, and C. 10%. The same data are shown for principal components 2 and 3 at a D. 1%, E. 5%, and F. 10% MAF threshold. Plots (G-I) use a minimum MAF threshold of 1% and color points by G. coverage, H. library preparation kit, and I. sequencer.



Supplemental Figure 14. Pairwise relatedness values plot for sample pairs captured at the same site. The mean relatedness between individuals was 0.



Supplemental Figure 15. R^2 values for pairs of SNPs on Chromosome 1 showing an average linkage block size of ~10 Kb.

Supplementary Table 1. Sites of sampling for this study.

Site Name	Latitude	Longitude
Crested Butte 1	38.818888	-106.89214
Crested Butte 2	38.857861	-106.959444
Mount Crested Butte	38.911722	-106.959194
Gothic 1	38.95525	-106.986916
Gothic 2	38.953055	-106.991666
Almont 1	38.678444	-106.825277
Almont 2	38.755138	-106.769555
Almont 3	38.731527	-106.74027
Almont 4	38.731777	-106.770777
Almont 5	38.723333	-106.7745
Almont 6	38.724416	-106.772333
Paonia	38.8575	-106.643833
Crawford	38.730277	-106.512555
Gunnison	38.586277	-106.892222
Washington Gulch	38.906324	-106.993627
Danni Ranch	38.7388	-106.8605

Supplementary Table 2. Summary of the distribution of library preparation kit and sequencing platform across all samples used in this study.

Library Preparation Kit	Sequencing Platform	Sample Size
Kapa	HiSeq 4000	7
Nextera	HiSeq 4000	27
Nextera	NovaSeq 6000	194
Seqwell	NovaSeq 6000	282
Total	-	510

Supplementary Table 3. Table depicting the distribution of male and female sampling for each site and region. Each site comes from one of the four listed regions, with Gothic having the majority of sampling.

Site	F	M
Almont1	22	40
Almont2	40	15
Almont3	12	7
Almont4	4	4
Almont5	11	15
Almont6	7	8
Crawford	13	23
CrestedButte1	2	0
CrestedButte2	2	7
DanniRanch	45	22
Gothic1	61	85
Gunnison	2	3
MCB	3	7
Washington Gulch	37	13
Region		
Almont	141	111
Crawford	13	23
Gothic	105	112
Gunnison	2	3

Supplementary Table 4. Models identifying predictors of *Ipomopsis* presence/absence. *Ipomopsis* was only common and evenly sampled at Gothic, so all models are limited to that site. A model was run to identify abiotic predictors, and phenotypic predictors across the summer.

<i>Ipomopsis</i> Abiotic Predictors				
Initial	(Sex + DoY) ² + (1 Year)			
Converged	DoY			
Trait	LR	Chisq	Df	Pr(>Chisq)
DoY	7.3974	1	0.006532	**
<i>Ipomopsis</i> Summer Model				
Initial	EC + C + CW + DoY + DoY*EC + DoY*CW + DoY*C			
Converged	EC + C + DoY + EC:DoY + C:DoY			
Trait	LR	Chisq	Df	Pr(>Chisq)
EC	7.7212	1	0.005458	**
C	2.7460	1	0.097501	.
DoY	9.0005	1	0.002699	**
EC:DoY	7.5111	1	0.006132	**
C:DoY	3.1874	1	0.074208	.

Supplementary Table 5. Models identifying predictors of *Mertensia* presence/absence. *Mertensia* was only common and evenly sampled at Gothic, so all models are limited to that site. A model was run to identify abiotic predictors, and phenotypic predictors across the summer.

<i>Mertensia</i> Abiotic Predictors				
Initial	(Sex + DoY) ² + (1 Year)			
Converged	Intercept			
Trait	LR	Chisq	Df	Pr(>Chisq)
DoY	13.738	1	2.10E-04	***
<i>Mertensia</i> Summer Model				
Initial	EC + C + CW + DoY + EC:DoY + CW:DoY + C:DoY			
Converged	EC + C + DoY + EC:DoY + C:DoY			
Trait	LR	Chisq	Df	Pr(>Chisq)
EC	3.8582	1	0.049504	*
C	9.4309	1	0.002134	**
DoY	5.2353	1	0.022133	*
EC:DoY	3.3813	1	0.06594	.
C:DoY	9.3758	1	0.002199	**

Supplementary Table 6. Models of changes in bill length over between the months of May and August in Gothic, Colorado. All models were first tested for annual variation as a random effect and sex or time of summer, along with interaction effects as fixed effects. Nonsignificant effects were first removed with a backwards stepwise function and predictors were evaluated with a type III ANOVA.

Full Gape over Summer					
Initial	$(\text{Sex} + \text{EL})^2 + (1 \text{Year})$				
Converged	Sex + EL				
Trait	Sum	Sq	Df	F	Pr(>F)
(Intercept)	17751	1	30543.5603	2.20E-16	***
Sex	22.5	1	38.6917	5.16E-09	***
EL	2.9	1	5.0038	0.02684	*
Residuals	83.1	143			
Transformed Exposed Culmen over Summer (Ordered Normalization)					
Initial	$(\text{Sex} + \text{EL})^2 + (1 \text{Year})$				
Converged	Sex + EL + (1 Year)				
Trait	Sum Sq	Df	F	Pr(>F)	
(Intercept)	6501.4886	1	2.20E-16	***	
Sex	39.2848	1	3.66E-10	***	
EL	5.0344	1	0.02485	*	

Supplementary Table 7. Confounding variables were determined for each phenotype using a backwards stepwise function and evaluated using a type III ANOVA. The appropriate transformation for each response variable is reported for each model and was determined using the bestNormalize package in R. Phenotypes were then transformed and models were evaluated with QQplots of the residuals.

Full Gape Covariates					
Initial	Wing Chord + Sex				
Converged	Wing Chord + Sex				
Transform	None				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	18.15606	1.39055	13.057	<2e-16	***
WC	0.06885	0.02738	2.515	0.0122	*
SexM	-0.81612	0.06009	-13.581	<2e-16	***
Multiple R ²	0.295	Adjusted R ²	0.2922		
Exposed Culmen Covariates					
Initial	Wing Chord + Sex				
Converged	Wing Chord + Sex				
Transform	None				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	14.44908	1.36878	10.556	<2e-16	***
WC	0.06273	0.02695	2.327	0.0203	*
SexM	-0.69868	0.05915	-11.812	<2e-16	***
Multiple R ²	0.2419	Adjusted R ²	0.2389		
Culmen Width Covariates					
Initial	Wing Chord + Sex				
Converged	Wing Chord + Sex				
Transform	Log				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	3.57425	1.54941	2.307	0.0215	*
WC	-0.05747	0.03051	-1.884	0.0602	.
SexM	-1.3671	0.06696	-20.418	<2e-16	***
Multiple R ²	0.455	Adjusted R ²	0.4528		
Curvature Covariates					
Initial	Exposed Culmen + Wing Chord + Sex				

Converged	Exposed Culmen + Sex				
Transform	BoxCox				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	-1.855	1.1632	-1.595	0.1114	
EC	0.1139	0.0659	1.728	0.0847	.
SexM	-0.2287	0.0998	-2.292	0.0223	*
Multiple R ²	0.03003	Adjusted R ²	0.0262		
Culmen Length/Culmen Width Ratio Covariates					
Initial	Wing Chord + Sex				
Converged	Wing Chord + Sex				
Transform	Log				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	-6.19188	1.84852	-3.35	0.00087	***
WC	0.11332	0.0364	3.113	0.00196	**
SexM	0.95712	0.07988	11.982	2.00E-16	***
Multiple R ²	0.2226	Adjusted R ²	0.2196		
Gape Width Covariates					
Initial	Wing Chord + Sex				
Converged	Wing Chord + Sex				
Transform	None				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	3.35882	0.65467	5.131	4.90E-07	***
WC	0.0414	0.01287	3.216	0.00143	**
SexM	-0.34421	0.02496	-13.79	2.00E-16	***
Multiple R ²	0.4016	Adjusted R ²	0.3981		
Culmen Depth Covariates					
Initial	Wing Chord + Sex				
Converged	Sex				
Transform	None				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	1.652769	0.006773	244.008	2.00E-16	***
SexM	-0.073646	0.010005	-7.361	1.43E-12	***
Multiple R ²	0.1396	Adjusted R ²	0.137		
Wing Chord Covariates					

Initial	Sex				
Converged	Sex				
Transform	None				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	50.78215	0.0615	825.737	2.00E-16	***
SexM	-0.46332	0.08794	-5.269	2.04E-07	***
Multiple R ²	0.0523	Adjusted R ²	0.05042		
Dorsal Shape PC1 Covariates					
Initial	Sex				
Converged	Sex				
Transform	YeoJohnson				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	0.33969	0.06934	4.899	1.55E-06	***
SexM	-0.78507	0.10541	-7.448	9.48E-13	***
Multiple R ²	0.1518	Adjusted R ²	0.149		
Dorsal Shape PC2 Covariates					
Initial	Sex				
Converged	Sex				
Transform	None				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	-0.000304	0.0002585	-1.177	0.24	
SexM	0.0005971	0.0003909	1.528	0.128	
Multiple R ²	0.007496	Adjusted R ²	0.004284		
Profile Shape PC1 Covariates					
Initial	Sex				
Converged	Sex				
Transform	Square Root				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	0.0009216	0.0005248	1.756	0.07966	.
SexM	-0.002198	0.0007534	-2.918	0.00368	**
Multiple R ²	0.01658	Adjusted R ²	0.01463		
Profile Shape PC2 Covariates					
Initial	Sex				
Converged	Sex				

Transform	Order Normalization				
Trait	Estimate	Std.	Error	t	Pr(> t)
(Intercept)	-0.12966	0.06139	-2.112	0.03517	*
SexM	0.26611	0.08795	3.026	0.00261	**
Multiple R ²	0.01774	Adjusted R ²	0.0158		

Supplementary Table 8. SNPs that passed the FDR significance threshold for univariate LMM GWAS along with the overlapping or nearest gene annotation.

Phenotype	Chromosome	Gene
Culmen Width	Chr1	RNF6
Culmen Depth	Chr14	IRPL2
Culmen L/W Ratio	Chr19	KSR2
Transformed DPC1	Chr2	M3K8
Gape Width	Chr2	CR021
Transformed DPC1	Chr3	BFSP1
Transformed Curvature	Chr7	CNTP5

Supplementary Table 9. SNPs that passed the FDR significance threshold for GWAS of Sex. The overlapping or nearest gene annotation are listed for chromosomes other than 4. All loci that are not significantly associated with sex in dorsal shape PC1 GWAS are highlighted in red and are considered false positive associations with sex.

Phenotype	Chromosome	Gene
Sex	Chr3	BROX
Sex	Chr3	AGRB3
Sex	Chr32	O14CZ
Sex	Chr32	ENV
Sex	Chr32	O14J1
Sex	Chr4	RUSC2
Sex	Chr4	GOLP3
Sex	Chr4	CTL1
Sex	Chr4	DYM
Sex	Chr4	CTIF
Sex	Chr4	NREP
Sex	Chr4	LRRC2
Sex	Chr4	DAPK1
Sex	Chr4	TLE1
Sex	Chr4	TSP4
Sex	Chr4	SNX2
Sex	Chr4	DTWD2
Sex	Chr4	SHOC1
Sex	Chr4	DNAI1
Sex	Chr4	F214B
Sex	Sca90	HDHD2

Supplementary Table 10. REML estimates of percent variance explained by typed genotypes using univariate LMM (chip heritability).

Phenotype	PVE	SE(PVE)
Culmen Depth	~0	4.92
Culmen Width	39.05	22.49
Transformed Culmen Width	35.02	23.18
Curvature	69.90	13.87
Transformed Curvature	62.02	16.69
Transformed Dorsal PC1	72.79	23.23
Exposed Culmen	64.47	24.88
Full Gape	54.80	26.08
Transformed Feather Count	99.98	2.79
Culmen L/W Ratio	48.70	27.05
Transformed Culmen L/W Ratio	46.70	26.34
Gape Width	41.63	30.72
Transformed Day of Year	39.90	18.31
Profile PC1	24.20	25.39
Wing Chord	33.62	22.38
Depth/Length Ratio	~0	2.90

Supplementary Table 11. Candidate loci from MLMM GWAS with p-value passing FDR-corrected threshold.

Phenotype 1	Phenotype 2	Gene
Profile PC1	Full Gape	CAD17
Exposed Culmen	Full Gape	ACOHC
Exposed Culmen	Full Gape	AGRA3
Exposed Culmen	Full Gape	CCG2
Exposed Culmen	Full Gape	CELF2
Exposed Culmen	Full Gape	CTR2
Exposed Culmen	Full Gape	DMTF1
Exposed Culmen	Full Gape	EGLN3
Exposed Culmen	Full Gape	F241A
Exposed Culmen	Full Gape	FBSL
Exposed Culmen	Full Gape	GALT9
Exposed Culmen	Full Gape	GDS1
Exposed Culmen	Full Gape	GNAT3
Exposed Culmen	Full Gape	GRM5
Exposed Culmen	Full Gape	KATL1
Exposed Culmen	Full Gape	KYNU
Exposed Culmen	Full Gape	LYN
Exposed Culmen	Full Gape	MACD2
Exposed Culmen	Full Gape	MCR
Exposed Culmen	Full Gape	MCU
Exposed Culmen	Full Gape	NECT3
Exposed Culmen	Full Gape	NTCP2
Exposed Culmen	Full Gape	PACRG
Exposed Culmen	Full Gape	PAR3L
Exposed Culmen	Full Gape	PEX5R
Exposed Culmen	Full Gape	PIEZ2
Exposed Culmen	Full Gape	PKN2
Exposed Culmen	Full Gape	PLD1
Exposed Culmen	Full Gape	PLPL9
Exposed Culmen	Full Gape	PO6F2
Exposed Culmen	Full Gape	POL

Exposed Culmen	Full Gape	RFOX1
Exposed Culmen	Full Gape	SMCA1
Exposed Culmen	Full Gape	SYFM
Exposed Culmen	Full Gape	SYNE2
Exposed Culmen	Full Gape	TEC
Exposed Culmen	Full Gape	THA
Exposed Culmen	Full Gape	TMC2
Exposed Culmen	Full Gape	TRI29
Exposed Culmen	Full Gape	UAP1
Exposed Culmen	Full Gape	VP37A
Exposed Culmen	Full Gape	WNK1
Exposed Culmen	Full Gape	WNT9A
Exposed Culmen	Full Gape	ZEB2
Culmen Depth	Profile Shape PC1	S12A7
Culmen Depth	Profile Shape PC1	LRAT1
Dorsal Shape PC1	Profile Shape PC1	PAK6
Dorsal Shape PC1	Profile Shape PC1	CD166
Dorsal Shape PC1	Profile Shape PC1	Z385D
Dorsal Shape PC1	Profile Shape PC1	GALT1
Dorsal Shape PC1	Profile Shape PC1	PACA
Dorsal Shape PC1	Profile Shape PC1	LRAT1
Profile Shape PC1	Culmen L/W Ratio	CLAP2
Profile Shape PC1	Culmen L/W Ratio	STXB4
Profile Shape PC1	Culmen L/W Ratio	LRAT1

Supplementary Table 12. Estimates of genetic correlation between phenotypes using MLMM in GEMMA.

Phenotype 1	Phenotype 2	Covariance	Vg 1	Vg 2	Correlation	Correlation
Culmen Width	Exposed Culmen	-0.245994	4.06601	6.62166	-0.04740857	-0.05
Culmen Width	Full Gape	2.05535	4.04588	5.48774	0.43619685	0.44
Culmen Width	Wing Chord	-2.02294	4.0273	7.31704	-0.372656153	-0.37
Curvature	Full Gape	2.18818	14.521	5.76227	0.239214764	0.24
Exposed Culmen	Full Gape	6.36841	6.54175	6.20122	0.999874458	1.00
Profile PC1	Curvature	-0.0195055	0.000355734	13.7707	-0.278686795	-0.28
Profile PC1	Exposed Culmen	-0.0251534	0.000504006	6.32205	-0.445604821	-0.45
Profile PC1	Full Gape	-0.0515572	0.000517724	5.20022	-0.993640571	-0.99
Culmen Width	Culmen Depth	1.765E-07	5.55468	8.33E-08	0.000259549	0.00
Culmen Depth	Profile PC1	1.86E-16	8.37E-08	6.68E-10	2.49046E-08	0.00
Dorsal PC1	Profile PC1	0.0623074	14.027	0.000276944	0.999680568	1.00
Full Gape	Wing Chord	1.8164	6.3791	8.2445	0.250466488	0.25
Curvature	F-Curvature	0.0053	13.2834	0.0001	0.14541903	0.15
Gape Width	Culmen Width	-0.023	4.5456	0.4747	-0.015657507	-0.02
Profile PC1	Culmen L/W	-0.0366	0.0005	8.5939	-0.558342819	-0.56
Gape Width	Dorsal PC1	0.824	0.5347	17.1901	0.271789652	0.27
Dorsal PC1	Culmen L/W	-7.2783	10.8515	14.5942	-0.578355565	-0.58
Culmen Depth	Exposed Culmen	0	0	8.1623	0	0.00

Supplementary Table 13. Hyperparameters estimated using BSLMM. h= the approximation to PVE explained by all loci. Rho = the proportion of genetic variance explained by sparse effect terms. Pi= proportion of variants with nonzero effects. Gamma = number of major effect loci.

Culmen Depth					LD -Pruned Culmen Depth					Difference
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	
h	0.3028603 28	0.26803125	0.015242708	0.76826051	h	0.343787385	0.3329973	0.0296432 17	0.7328 52568	0.0409270 57
PVE	0.2482379 99	0.2104376	0.009357142	0.696116743	PVE	0.294095136	0.2811378	0.0213045 4	0.6647 004	0.0458571 37
rho	0.5950207 81	0.6320521	0.055750659	0.982451753	rho	0.667675587	0.7148595	0.1058666	0.9877 733	0.0726548 06
PGE	0.4739530 52	0.48318415	0	0.968849903	PGE	0.596562253	0.63158675	0.0278907 42	0.9820 13125	0.1226092
pi	9.05E-07	3.01E-07	4.77E-08	7.70E-06	pi	1.12E-06	7.85E-07	6.00E-08	3.69E- 06	2.10419E- 07
n.gamma	22.00829	8	0	191	n.gamma	27.3389	20	1	89	5.33061
Culmen Width					LD -Pruned Culmen Width					Difference
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	
h	0.4131074 08	0.404999	0.05253937	0.8254574	h	0.38520275	0.3698627	0.0287782 96	0.8404 976	0.0279046 59
PVE	0.3745343 88	0.35949385	0.039649638	0.799077603	PVE	0.326493427	0.3025954	0.0175139 3	0.7860 3907	0.0480409 61
rho	0.4202062 09	0.39369435	0.032853809	0.92843032	rho	0.362613703	0.30949485	0.0163713 57	0.9260 802	0.0575925 06
PGE	0.3209372 32	0.27721515	0.000660215	0.8846675	PGE	0.1817358	0.11252805	0	0.7894 71053	0.1392014 32
pi	2.80E-06	2.36E-06	5.72E-08	7.47E-06	pi	8.19E-07	2.49E-07	4.35E-08	3.80E- 06	1.98156E- 06
n.gamma	69.03421	59	1	183	n.gamma	19.57689	6	0	91	49.45732
Curvature					LD -Pruned Curvature					Difference
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	
h	0.6471121 7	0.668206	0.246039498	0.9267766	h	0.671247168	0.69123165	0.2778051	0.9521 78205	0.0241349 98
PVE	0.6152350 64	0.6339398	0.212043363	0.908768455	PVE	0.646129179	0.66394095	0.2441849	0.9463 25503	0.0308941 15
rho	0.2256996 11	0.18575205	0.010155715	0.69376631	rho	0.282300907	0.2639602	0.0237070 9	0.6789 95585	0.0566012 96
PGE	0.1061712 5	0.08528821	0	0.338817508	PGE	0.190635921	0.1715143	0.0005679 68	0.5144 299	0.0844646 71
pi	1.12E-06	6.56E-07	4.49E-08	3.94E-06	pi	4.47E-06	3.26E-06	5.66E-08	1.21E- 05	3.35024E- 06
n.gamma	26.98663	16	0	98	n.gamma	109.44374	79	1	294	82.45711
Dorsal Shape PC1					LD -Pruned Dorsal Shape PC1					Difference
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	

h	0.7092845 08	0.751864	0.178474288	0.99202694	h	0.700221579	0.7400218	0.1794919 1	0.9911 0331	0.0090629 29
PVE	0.6749153 21	0.7112818	0.14443975	0.989468853	PVE	0.670980122	0.70562015	0.1466587	0.9888 703	0.0039351 99
rho	0.2376668 88	0.17539465	0.00612882	0.78294354	rho	0.227833312	0.1656603	0.0061474 52	0.7698 89463	0.0098335 77
PGE	0.0827463 41	0.04519105	0	0.372938358	PGE	0.096985249	0.055730605	0	0.4492 51267	0.0142389 09
pi	5.72E-07	1.71E-07	4.32E-08	3.68E-06	pi	5.41E-07	2.65E-07	4.45E-08	2.56E- 06	3.12226E- 08
n.gamma	13.19378	4	0	91	n.gamma	12.53593	6	0	63	0.65785
Culmen L/W					LD -Pruned Culmen L/W					
Hyperpar ameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.5570346 71	0.5650458	0.109737	0.953822625	h	0.55926143 7	0.5728334	0.0870246 54	0.9632 8	0.0022267 66
PVE	0.5207802 92	0.5213894	0.089729088	0.9466648	PVE	0.52331372 3	0.5276015 5	0.0661699 1	0.9580 26903	0.0025334 31
rho	0.3911508 81	0.367273	0.036644794	0.8891768	rho	0.33840688 4	0.3056549	0.0235103 06	0.8551 0919	0.0527439 97
PGE	0.2948260 9	0.2625832	0.000373157	0.82725236	PGE	0.23026668 2	0.1913382	0	0.7499 13025	0.0645594 08
pi	2.82E-06	1.67E-06	5.46E-08	1.11E-05	pi	1.30E-06	9.15E-07	5.53E-08	5.31E- 06	1.51968E- 06
n.gamma	69.83838	41	1	282	n.gamma	31.54534	22	0	130	38.29304
Gape Width					LD -Pruned Gape Width					
Hyperpar ameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.6255081 54	0.63003025	0.2915276	0.925513173	h	0.54390696 7	0.5525567	0.1116573	0.9215 077	0.0816011 87
PVE	0.5752180 75	0.56818075	0.26182512	0.910122015	PVE	0.49683992 7	0.4939124 5	0.0891676 69	0.9046 394	0.0783781 49
rho	0.6881358 12	0.69990965	0.30532923	0.979043613	rho	0.50690305 8	0.5022658 5	0.0764357 97	0.9470 89865	0.1812327 54
PGE	0.6246824 21	0.6208421	0.251121255	0.9706436	PGE	0.40729099 5	0.3836412	0.0067517 07	0.9171 06683	0.2173914 26
pi	2.56E-06	2.46E-06	2.61E-07	5.67E-06	pi	1.00E-06	5.89E-07	5.61E-08	4.15E- 06	1.55235E- 06
n.gamma	63.11593	62	8	136	n.gamma	24.58297	15	1	101	38.53296
Profile PC1					LD -Pruned Profile PC1					
Hyperpar ameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.3385097 94	0.31493355	0.016919717	0.7945728	h	0.41118559 1	0.4072309 5	0.0398291 55	0.8270 133	0.0726757 98
PVE	0.2880100 97	0.2568783	0.011307992	0.73288418	PVE	0.36647277 1	0.3503917 5	0.0315761 2	0.7953 84517	0.0784626 74
rho	0.3766915 57	0.32651495	0.0162162	0.935336235	rho	0.48598446 6	0.480647	0.0427565 5	0.9530 05358	0.1092929 09
PGE	0.2236376 05	0.1547167	0	0.844404335	PGE	0.39291709 7	0.3616967	0.0162978 19	0.9226 284	0.1692794 92

pi	8.40E-07	4.67E-07	4.58E-08	4.14E-06	pi	5.18E-06	5.27E-06	7.47E-08	1.22E-05	4.33707E-06
n.gamma	20.00012	11	0	101	n.gamma	127.10352	131	2	294	107.1034
Profile PC2					LD -Pruned Profile PC2					
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.1795567 79	0.13812335	0.005670327	0.575166468	h	0.17017753 1	0.1298925 5	0.0048667 1	0.5559 55373	0.0093792 49
PVE	0.1272171 58	0.095299725	0.00329981	0.41728321	PVE	0.12592162	0.0934144 1	0.0031381 89	0.4180 1067	0.0012955 38
rho	0.4629982 51	0.4423155	0.01993637	0.971244623	rho	0.44621081 6	0.4164127 5	0.0189401 74	0.9677 817	0.0167874 35
PGE	0.2659824 83	0.1746306	0	0.910964057	PGE	0.27349694 8	0.1885895	0	0.9134 15988	0.0075144 64
pi	7.68E-07	3.10E-07	4.42E-08	4.05E-06	pi	1.05E-06	4.65E-07	4.48E-08	5.78E-06	2.78325E-07
n.gamma	18.33473	7	0	101	n.gamma	25.06729	11	0	142	6.73256
Day of Year					LD -Pruned Day of Year					
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.4801695 72	0.48904305	0.1001843	0.822929	h	0.81033308 5	0.8294875	0.5329466 08	0.9796 01225	0.3301635 13
PVE	0.4026101 51	0.40245065	0.073679455	0.740260578	PVE	0.75885244 6	0.7759319	0.4540723 78	0.9716 75583	0.3562422 95
rho	0.4494909 33	0.43750885	0.034380176	0.9248928	rho	0.60326627	0.6035294 5	0.3217377 13	0.8860 374	0.1537753 37
PGE	0.2705371 05	0.2257786	0	0.810808988	PGE	0.46678491	0.4436109	0.2907217 8	0.7760 8835	0.1962478 04
pi	3.90E-07	2.18E-07	4.46E-08	1.74E-06	pi	5.05E-07	4.63E-07	1.73E-07	1.03E-06	1.14692E-07
n.gamma	9.41211	5	0	44	n.gamma	12.67113	12	6	24	3.25902
Dorsal Shape PC2					LD -Pruned Dorsal Shape PC2					
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.2841240 64	0.234653 2	0.010704449	0.812778077	h	0.3140853 46	0.2694894 5	0.0139046 02	0.84661104	0.0299 61283
PVE	0.2375664 02	0.185309 9	0.007027248	0.7535078	PVE	0.2770859 15	0.2264015	0.0097177 79	0.813030315	0.0395 19513
rho	0.4482072 75	0.420427 6	0.01950416	0.965476358	rho	0.4640673 28	0.4439829	0.0258914 27	0.966145093	0.0158 60053
PGE	0.3087668 8	0.231850 35	0	0.932493385	PGE	0.3599788 83	0.3051583	0	0.946754478	0.0512 12003
pi	9.14E-07	3.07E-07	4.48E-08	4.60E-06	pi	1.53E-06	6.50E-07	4.63E-08	7.57E-06	6.1492 5E-07
n.gamma	21.87506	7	0	108	n.gamma	36.74665	15	0	185	14.871 59
Exposed Culmen					LD -Pruned Exposed Culmen					
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference

h	0.5919725 46	0.627015	0.071063514	0.95415669	h	0.5938080 15	0.6303618	0.0657187 09	0.956382768	0.0018 35469
PVE	0.5473566 49	0.573496	0.04853948	0.9362165	PVE	0.5540582 1	0.5838471 5	0.0477168 54	0.94272839	0.0067 01561
rho	0.2400334 12	0.167331 8	0.006076838	0.825351335	rho	0.2188975 07	0.1428607	0.0050828 07	0.80922021	0.0211 35905
PGE	0.0821247 98	0.043789 355	0	0.43535975	PGE	0.0764479 8	0.0387754 75	0	0.40612066	0.0056 76818
pi	6.09E-07	2.68E-07	4.32E-08	3.04E-06	pi	9.14E-07	3.19E-07	4.37E-08	4.18E-06	3.0568 3E-07
n.gamma	14.12541	6	0	74	n.gamma	21.64925	7	0	102	7.5238 4
F-Curvature					LD -Pruned F-Curvature					
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.4426995 63	0.439560 65	0.031775547	0.89776442	h	0.4045601 18	0.3929477 5	0.0215763 01	0.884053995	0.0381 39445
PVE	0.4049207 48	0.395840 05	0.022323512	0.873469237	PVE	0.3802497 81	0.3655567	0.0172436 57	0.86519978	0.0246 70968
rho	0.2893588 59	0.229995 2	0.010241722	0.87690071	rho	0.2445254 29	0.1747340 5	0.0072980 53	0.852128257	0.0448 3343
PGE	0.1664416 35	0.107485 3	0	0.726313245	PGE	0.1587092 08	0.0959223 05	0	0.7343863	0.0077 32427
pi	2.29E-06	1.08E-06	4.58E-08	1.01E-05	pi	5.84E-06	6.38E-06	5.43E-08	1.25E-05	3.5504 6E-06
n.gamma	56.08317	27	0	249	n.gamma	142.70369	157	0	297	86.620 52
Full Gape					LD -Pruned Full Gape					
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.4858374 77	0.503959 3	0.027765781	0.90526713	h	0.4992648 73	0.5182789 5	0.0346182 71	0.914167628	0.0134 27396
PVE	0.4390107 05	0.449271 5	0.018924861	0.868443903	PVE	0.4679480 89	0.4810999	0.0263372 5	0.89700238	0.0289 37384
rho	0.2532716 1	0.167212 3	0.005692605	0.86775924	rho	0.2311843 98	0.1617495	0.0072306 29	0.8272223	0.0220 87212
PGE	0.0951892 29	0.04304	0	0.583706785	PGE	0.1247848 09	0.0753955 4	0	0.6237076	0.0295 9558
pi	1.26E-06	3.29E-07	4.32E-08	7.38E-06	pi	2.87E-06	2.08E-06	4.70E-08	1.10E-05	1.6128 6E-06
n.gamma	30.15789	7	0	178	n.gamma	69.80292	51	0	267	39.645 03
Feather Count					LD -Pruned Feather Count					
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	Difference
h	0.6356122 56	0.698746 75	0.04373997	0.9991194	h	0.6268784 36	0.6866174 5	0.0405038 14	0.9989555	0.0087 33821
PVE	0.6325286 47	0.696905 05	0.039757748	0.9991636	PVE	0.6167216 01	0.6750006 5	0.0329275 38	0.998933	0.0158 07047
rho	0.4076051 31	0.367009 55	0.017996929	0.951795208	rho	0.3943192 34	0.3501871	0.0148925 1	0.94812961	0.0132 85897

PGE	0.3846593 68	0.334648 95	0	0.956706158	PGE	0.3453729 34	0.2775692	0	0.949166675	0.0392 86434
pi	1.99E-06	1.09E-06	6.28E-08	9.70E-06	pi	1.73E-06	7.27E-07	5.53E-08	8.04E-06	2.5745 E-07
n.gamma	40.04394	22	0	197	n.gamma	34.87126	14	0	162	5.1726 8
Wing Chord					LD -Pruned Wing Chord					
Hyperparameter	mean	median	2.50%	97.50%		mean	median	2.50%	97.50%	
h	0.3576035 01	0.34952775	0.030383461	0.7392574	h	0.3274158 51	0.313278	0.0222452 48	0.7160967	0.030187 651
PVE	0.3266440 79	0.3136877	0.024397	0.707782935	PVE	0.3039464 41	0.2864062	0.0177065 73	0.6907957	0.022697 638
Rho	0.3686912 04	0.3273823	0.017699102	0.9201934	rho	0.3181236 09	0.2625246 5	0.0132429 95	0.8987354	0.050567 595
PGE	0.2818472 72	0.2240739	0.003647392	0.873957	PGE	0.2418897 91	0.1771841	0.0032400 55	0.840181917	0.039957 48
Pi	4.41E-06	4.34E-06	7.49E-08	1.04E-05	pi	5.60E-06	6.15E-06	7.76E-08	1.17E-05	1.19557E -06
n.gamma	108.26809	108	1	253	n.gamma	137.21065	153	1	286	28.94256

Supplementary Table 14. Candidate loci from BSLMM GWAS in the top 0.01% of effect sizes or a posterior inclusion probability above 10%.

Phenotype	SNP ID	Beta	Gene
Culmen Depth	Chr1_106000249	1.52E-12	NUD4B
Culmen Depth	Chr1_139761274	1.53E-12	TY3H
Culmen Depth	Chr1_39105000	1.26E-12	MZT1
Culmen Depth	Chr1_58111195	1.14E-12	T255B
Culmen Depth	Chr1_80127371	1.13E-12	DMD
Culmen Depth	Chr1_85045171	1.32E-12	DIK2B
Culmen Depth	Chr18_2267195	1.43E-12	ITCH
Culmen Depth	Chr18_8130746	1.25E-12	GAG
Culmen Depth	Chr18_902453	1.24E-12	DLGP4
Culmen Depth	Chr19_7839304	1.36E-12	SLIK4
Culmen Depth	Chr2_127408945	1.39E-12	MATN2
Culmen Depth	Chr2_85452679	1.15E-12	NDUS6
Culmen Depth	Chr29_2647570	1.17E-12	ACE
Culmen Depth	Chr5_32508027	7.79E-13	ARAP2
Culmen Depth	Chr6_19515864	1.51E-12	ABCD4
Culmen Depth	Chr7_16985213	1.41E-12	TANK
Culmen L/W	Chr1_150119423	2.13E-27	RERGL
Culmen L/W	Chr1_18085705	2.17E-27	SGCG
Culmen L/W	Chr11_3644548	8.70E-28	PCD10
Culmen L/W	Chr16_5154919	1.62E-27	RS13
Culmen L/W	Chr2_45282165	1.96E-27	GLI3
Culmen L/W	Chr2_48885118	1.77E-27	HERP2
Culmen L/W	Chr2_74169731	9.15E-28	POL
Culmen L/W	Chr24_2872626	1.98E-27	CIDEC
Culmen L/W	Chr3_32621230	1.81E-27	UTRN
Culmen L/W	Chr3_45424960	2.22E-27	FERM1
Culmen L/W	Chr3_76066854	1.85E-27	CD109
Culmen L/W	Chr3_82664429	2.21E-27	M3K7
Culmen L/W	Chr3_86800925	2.14E-27	POL
Culmen L/W	Chr3_99827389	1.48E-27	E2F6
Culmen L/W	Chr5_7591454	2.03E-27	GRID2

Culmen L/W	Chr6_9662746	2.13E-27	RA51B
Culmen L/W	Chr8_21477314	2.21E-27	P4HA1
Culmen Width	Chr1_130113573	1.68E-68	NP1L1
Culmen Width	Chr1_193965136	6.28E-69	PLXA4
Culmen Width	Chr1_40071896	1.17E-68	TBCD4
Culmen Width	Chr1_75675298	7.21E-69	SH3K1
Culmen Width	Chr1_76973770	9.64E-69	PHEX
Culmen Width	Chr1_80812055	6.38E-69	DMD
Culmen Width	Chr1_95170560	1.70E-68	NCAM2
Culmen Width	Chr12_9283083	2.29E-68	PLXB1
Culmen Width	Chr14_12345008	2.31E-68	TM9S2
Culmen Width	Chr2_144147695	9.78E-69	COMA1
Culmen Width	Chr2_9610675	3.06E-70	CNDG2
Culmen Width	Chr3_6540908	1.27E-68	RYR2
Culmen Width	Chr4_66497423	2.23E-68	CSPG2
Culmen Width	Chr5_26059345	4.45E-69	TRM9B
Culmen Width	Chr5_2947288	2.33E-68	PAPS1
Culmen Width	Chr7_28334441	3.48E-69	PAR3L
Culmen Width	Chr7_7463522	2.06E-68	LYPD1
Curvature	Chr1_115156556	0.000402637	LSAMP
Curvature	Chr1_147106570	0.000406908	NMDE2
Curvature	Chr11_17047230	0.000416896	NKX11
Curvature	Chr12_5876792	0.000419663	SAMC
Curvature	Chr15_10631902	0.000411289	CNOT6
Curvature	Chr17_4832457	0.000419941	KNOP1
Curvature	Chr2_111147424	0.000409526	PENK
Curvature	Chr28_1071658	0.00041493	GBG7
Curvature	Chr3_18086737	0.000416932	SIX3
Curvature	Chr31_388638	0.00041556	IL6RA
Curvature	Chr4_35381122	0.000420049	GNAQ
Curvature	Chr6_26333772	0.000408468	GLUCM
Curvature	Chr7_20340986	0.000418874	DCE1
Curvature	Chr7_34609618	0.000419426	PLCL1
Curvature	Chr8_23133007	0.000419749	RUFY2
Day of Year	Chr1_127837014	0.000360178	ACSS3

Day of Year	Chr1_129414246	0.000359526	NAV3
Day of Year	Chr1_184869074	0.000358309	GNAI1
Day of Year	Chr1_29133360	0.000348822	CC122
Day of Year	Chr1_57542895	0.000350508	AT11A
Day of Year	Chr10_6140496	0.00035123	SENP5
Day of Year	Chr12_11605593	0.000358436	RPN1
Day of Year	Chr2_3159423	0.000342318	VIPR
Day of Year	Chr20_3264556	0.000359331	PSD12
Day of Year	Chr3_107530797	0.000343254	OPN5
Day of Year	Chr3_12242818	0.000360078	LTBP1
Day of Year	Chr3_32223990	0.000359907	SHPRH
Day of Year	Chr3_44999813	0.000360334	HAOX1
Day of Year	Chr9_6368743	0.000349038	FAF1
Dorsal PC1	Chr1_153742238	9.32E-13	RASK
Dorsal PC1	Chr1_65028464	9.98E-13	P
Dorsal PC1	Chr10_8835250	1.03E-12	GEMC1
Dorsal PC1	Chr11_17467303	9.62E-13	CTBP1
Dorsal PC1	Chr2_37824398	1.03E-12	NR1D2
Dorsal PC1	Chr2_40213028	7.46E-13	RBMS3
Dorsal PC1	Chr2_68856246	6.74E-13	CAD20
Dorsal PC1	Chr33_781892	5.37E-13	SPAS2
Dorsal PC1	Chr4_3602335	9.49E-13	SIG15
Dorsal PC1	Chr4_67494516	1.01E-12	COX7C
Dorsal PC1	Chr5_8138944	7.79E-13	CCSE1
Dorsal PC1	Chr7_25247636	9.53E-13	RAPH1
Dorsal PC1	Chr8_1151214	7.85E-13	MGMT
Dorsal PC1	Chr9_1515165	9.20E-13	LEPR
Exposed Culmen	Chr1_153443713	9.62E-31	GYS2
Exposed Culmen	Chr1_167478764	7.59E-31	PZRN4
Exposed Culmen	Chr1_171704938	9.47E-31	MET
Exposed Culmen	Chr1_38484491	9.18E-31	DACH1
Exposed Culmen	Chr1_60686753	7.16E-31	P3F3B
Exposed Culmen	Chr1_70313535	8.75E-31	GP143
Exposed Culmen	Chr14_11171908	4.49E-31	PCD19
Exposed Culmen	Chr14_5342086	8.53E-31	GRIA3

Exposed Culmen	Chr2_38825153	9.64E-31	LRC3B
Exposed Culmen	Chr25_4205946	9.65E-31	CEPU1
Exposed Culmen	Chr3_103065311	8.94E-31	5NT1B
Exposed Culmen	Chr3_11643975	9.39E-31	RMD2
Exposed Culmen	Chr3_28520776	8.71E-31	SCAF8
Exposed Culmen	Chr4_11075143	8.75E-31	MTMRC
Exposed Culmen	Chr6_36207923	9.17E-31	KCNH5
Exposed Culmen	Chr7_33182388	9.10E-31	TEFF2
F-Curvature	Chr1_112213271	0.000102766	WDR3
F-Curvature	Chr1_118373157	0.000105865	TOM70
F-Curvature	Chr1_125443973	0.000105167	MGT4C
F-Curvature	Chr1_150151510	9.84E-05	RERGL
F-Curvature	Chr1_163916597	0.000100562	UBP15
F-Curvature	Chr1_67160118	9.59E-05	ZBED1
F-Curvature	Chr1_92664058	0.000104487	AT55
F-Curvature	Chr10_3304104	0.000104305	NLGN1
F-Curvature	Chr12_13458039	9.80E-05	SELK
F-Curvature	Chr15_9460361	9.50E-05	CPLX2
F-Curvature	Chr16_14800032	9.89E-05	COR1B
F-Curvature	Chr2_18952504	0.000104617	MALR1
F-Curvature	Chr2_21557427	0.000100894	Z804B
F-Curvature	Chr2_23105982	0.000105309	SAMD9
F-Curvature	Chr21_6960489	0.000105948	EFCB5
F-Curvature	Chr22_8619544	9.91E-05	TF3C5
F-Curvature	Chr3_29779830	0.000102169	ESR1
F-Curvature	Chr3_85140442	0.000105949	ASGL1
Full Gape	Chr1_143329073	3.73E-39	MYH9
Full Gape	Chr1_160739728	2.86E-39	KCNA6
Full Gape	Chr1_174007309	3.32E-39	CAPS2
Full Gape	Chr1_176816084	3.70E-39	I17EL
Full Gape	Chr1_70301142	3.67E-39	GP143
Full Gape	Chr1_73442359	3.51E-39	MSPD2
Full Gape	Chr11_4622400	3.45E-39	XCT
Full Gape	Chr14_16650430	3.71E-39	AFF2
Full Gape	Chr2_22027260	3.70E-39	PTTG

Full Gape	Chr2_44886558	3.34E-39	RM32
Full Gape	Chr25_1197422	3.70E-39	NCAM1
Full Gape	Chr3_17498720	3.70E-39	SRBD1
Full Gape	Chr3_26409894	2.44E-39	BROX
Full Gape	Chr3_46231141	2.34E-39	BC11A
Full Gape	Chr4_56042502	2.47E-39	DHB4
Full Gape	Chr5_21851770	3.24E-39	HELT
Full Gape	Chr9_1107939	3.73E-39	DIRA2
Gape Width	Chr1_103352167	2.35E-37	IGSF2
Gape Width	Chr10_19106904	1.80E-37	DUS28
Gape Width	Chr10_9626399	3.54E-37	AT134
Gape Width	Chr14_4215653	2.83E-37	SMCA1
Gape Width	Chr17_10436695	1.86E-38	MD1L1
Gape Width	Chr19_2773981	3.87E-37	RTN4R
Gape Width	Chr2_129053749	3.75E-37	RIR2B
Gape Width	Chr2_140675020	1.80E-37	EFR3A
Gape Width	Chr2_26427380	3.03E-37	PHF14
Gape Width	Chr2_65317660	3.42E-37	SYFM
Gape Width	Chr2_70536670	3.36E-37	CADH9
Gape Width	Chr4_17417284	1.01E-37	ARL15
Gape Width	Chr6_42763618	1.10E-37	RTXE
Gape Width	Chr7_7590045	6.80E-38	S35F5
Gape Width	Chr8_26607627	5.69E-38	CCSE2
Profile PC1	Chr1_105359339	0.003605865	PGS2
Profile PC1	Chr1_120353354	0.003706532	CD166
Profile PC1	Chr1_24020984	0.003651516	POSTN
Profile PC1	Chr1_49524430	0.003683276	H6ST3
Profile PC1	Chr11_21536366	0.003714615	GFRA4
Profile PC1	Chr13_1053777	0.003454688	CSPG4
Profile PC1	Chr2_142694367	0.003714645	KHDR3
Profile PC1	Chr2_41065637	0.003690153	CKLF6
Profile PC1	Chr21_6537362	0.003574022	NEK8
Profile PC1	Chr22_1918384	0.003681849	RABL6
Profile PC1	Chr3_93859558	0.003700014	DLGP2
Profile PC1	Chr5_25861718	0.003674407	RHG07

Profile PC1	Chr5_37275491	0.003691039	LCORL
Profile PC1	Chr8_21333635	0.003600035	IPMK
Profile PC1	Chr9_25296969	0.003618481	BRNP3
Profile PC1	Chr9_28515071	0.003710082	TEDC1
Profile PC1	Chr9_4877727	0.003714528	ACO11
Wing Chord	Chr1_138242318	2.09E-07	AK1D1
Wing Chord	Chr1_178366705	2.10E-07	POL
Wing Chord	Chr1_28656996	2.09E-07	T22D1
Wing Chord	Chr1_32621368	1.88E-07	PCD17
Wing Chord	Chr1_57760454	1.86E-07	PCID2
Wing Chord	Chr1_76876446	1.89E-07	PHEX
Wing Chord	Chr10_22686466	1.91E-07	DAW1
Wing Chord	Chr10_8771697	2.08E-07	IL1AP
Wing Chord	Chr11_7505820	2.10E-07	PO4F2
Wing Chord	Chr12_11983322	2.08E-07	FLNB
Wing Chord	Chr12_16267918	1.84E-07	CISH
Wing Chord	Chr18_5762478	2.07E-07	MYBB
Wing Chord	Chr2_2717149	1.79E-07	OBSCN
Wing Chord	Chr2_91593388	2.10E-07	FBX15
Wing Chord	Chr2_95427798	1.79E-07	ACTHR
Wing Chord	Chr22_5047426	2.02E-07	NTM1A
Wing Chord	Chr3_107438853	1.91E-07	PTHD4
Wing Chord	Chr4_54787972	1.95E-07	KC1G3
Wing Chord	Chr9_14291375	1.88E-07	KAPCB
Wing Chord	Chr9_6907662	1.58E-07	ELAV4
Phenotype	SNP ID	Beta	Gene
Feather Count	Chr1_57251693	0.00584373	GCP3
Feather Count	Chr1_68642250	0.002701956	NLGNX
Feather Count	Chr7_20339953	0.005944166	DCE1
Feather Count	Chr9_15671185	0.012780378	PKN2
Feather Count	Chr9_6829396	0.004464215	ELAV4
Feather Count	Chr1_117595438	0.011835565	TIPRL
Feather Count	Chr1_49595835	0.029140122	H6ST3
Feather Count	Chr10_7184900	0.029189001	ARMC9
Feather Count	Chr13_6358326	0.00313314	GCNT3

Feather Count	Chr13_8158411	0.033894632	BBS4
Feather Count	Chr15_2612676	0.004280068	S4A4
Feather Count	Chr2_80706385	0.013113597	COBL
Feather Count	Chr2_137885726	0.022203055	TMM65
Feather Count	Chr21_8699041	0.002336208	TBX2
Feather Count	Chr24_6225656	0.018806688	CARL2
Feather Count	Chr1_191945319	0.003939236	ITIH5

Supplementary Table 15. Genetic variances and correlations between trait pairs in a multivariate LMM GWAS for bill length, arrival time, and *Ipomopsis* presence/absence. All candidates passing the FDR-correction threshold are listed in the last panel.

Phenotype	Ipomopsis	Length	Arrival
Vg	2.48625E-06	7.0716	9.86736

Phenotype Pairs	I*L	I*A	A*L
Genetic Correlation	-0.002128368	-0.0045886	0.46325869

Gene				
NKX24	O14CZ	CTR2	P4HA1	MYO16
GRM5	DYM	SH3K1	PKN2	NLGN1
ESR1	GNAQ	ZEB2	WNK1	DAW1
DLGP2	LRRC2	DCE1	TY3H	PEX5R
PNOC	SHOC1	S35F5	H6ST3	DUS28
O14J1	CCSE1	RAPH1	POL	GBRA5
RPN1	ATS5	VIPR	COMA1	I17EL
CISH	M3K8	SYFM	TBX2	THA
GCNT3	MGT4C	PENK	TF3C5	

Supplementary Table 16. Candidate locus pairs identified above the 95th percentile for pairwise LD. Proteins listed are based on best hit alignments to the Uniprot database using Diamond. Candidate pairs were subset based on whether LD between SNPs was interchromosomal or greater than 2Mb. All pairwise LD values for Chromosomes 4 and 32 were excluded.

95th percentile				
Chr1:92664058	Chr3:29779830	0.041108	ATS5	ESR1
Chr7:20339953	Chr8:21477314	0.041458	DCE1	P4HA1
Chr1:134160980	Chr7:3338064	0.041842	WNK1	ZEB2
Chr1:7989546	Chr3:29779830	0.042287	GRM5	ESR1
Chr1:75675298	Chr7:20339953	0.042697	SH3K1	DCE1
Chr1:92664058	Chr7:20339953	0.043147	ATS5	DCE1
Chr2:111147424	Chr10:19106904	0.043316	PENK	DUS28
Chr3:29779830	Chr3:93859558	0.043412	ESR1	DLGP2
Chr1:178366705	Chr9:15773099	0.044337	POL	PKN2
Chr1:92664058	Chr13:6358326	0.04478	ATS5	GCNT3
Chr1:64370001	Chr9:15773099	0.045171	GBRA5	PKN2
Chr1:75675298	Chr7:7590045	0.045212	SH3K1	S35F5
Chr1:178366705	Chr2:144147695	0.045268	POL	COMA1
Chr1:49524430	Chr3:29779830	0.046035	H6ST3	ESR1
Chr7:20339953	Chr10:3304104	0.046238	DCE1	NLGN1
Chr2:144147695	Chr5:24304015	0.046663	COMA1	CTR2
Chr1:178366705	Chr7:7590045	0.047348	POL	S35F5
Chr7:3338064	Chr8:21477314	0.04751	ZEB2	P4HA1
Chr1:7989546	Chr2:144147695	0.048681	GRM5	COMA1
Chr9:15773099	Chr10:4807429	0.048858	PKN2	PEX5R
Chr1:7989546	Chr13:6358326	0.049159	GRM5	GCNT3
Chr1:75675298	Chr1:139761274	0.049968	SH3K1	TY3H
Chr1:7989546	Chr3:700429	0.050116	GRM5	NKX24
Chr1:134160980	Chr3:93859558	0.051113	WNK1	DLGP2

Chr1:134160980	Chr1:139761274	0.051209	WNK1	TY3H
Chr2:111147424	Chr2:144147695	0.052091	PENK	COMA1
Chr1:7989546	Chr1:75675298	0.053236	GRM5	SH3K1
Chr3:29779830	Chr12:11605593	0.054167	ESR1	RPN1
Chr1:7989546	Chr1:49524430	0.05465	GRM5	H6ST3
Chr5:8138944	Chr12:16267918	0.054694	CCSE1	CISH
Chr2:111147424	Chr8:21477314	0.055441	PENK	P4HA1
Chr1:7989546	Chr12:16267918	0.056932	GRM5	CISH
Chr1:55486662	Chr5:24304015	0.057662	MYO16	CTR2
Chr1:176816084	Chr3:29779830	0.058771	I17EL	ESR1
Chr3:700429	Chr7:3338064	0.059449	NKX24	ZEB2
Chr1:139761274	Chr7:20339953	0.063811	TY3H	DCE1
Chr3:29779830	Chr5:8138944	0.069805	ESR1	CCSE1
Chr1:75675298	Chr8:21477314	0.071222	SH3K1	P4HA1
Chr2:111147424	Chr7:20339953	0.073405	PENK	DCE1
Multiple Contacts >2Mb		Multiple Chromosomes		
ATS5		ATS5		
CCSE1		CCSE1		
COMA1		CISH		
DCE1		COMA1		
ESR1		DCE1		
GRM5		ESR1		
H6ST3		GCNT3		
NKX24		GRM5		
PENK		NKX24		
PKN2		P4HA1		
SH3K1		PENK		
TY3H		S35F5		
WNK1		SH3K1		

ZEB2	ZEB2
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Abstract

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Chapter 3

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