American Kestrel Genoscape Project

University of California Los Angeles, Boise State University, HawkWatch International, the American Kestrel Partnership

FEATHER SAMPLING PROTOCOL

Our plan - We have completed genomic sequencing, and are developing primers for assays of hundreds of samples. We will be building population specific migration maps within the next couple of years for American kestrels. Please help us collect samples from American kestrels across their range and annual cycle.

Our request - We welcome feathers from **migratory or resident kestrels, collected any time of the year, including breeding, migration, or winter.** When body feathers are pulled, a small amount of skin cells remain attached to the quill of the feather. These skin cells are a valuable source of DNA that can be used to determine the population origin of an individual bird. We recommend that two body feathers be collected during the banding process from each bird. There is no need to collect feathers from the same individual more than once. And there is no need to collect samples from every nestling in a brood. One nestling will suffice.

Necessary equipment and required permits

To collect feathers you will need small envelopes (coin envelopes work great) and a fine tip pen.

In the US, to collect 2 body feathers from American kestrels, banders will need a federal bird banding permit from the **USGS Bird Banding Laboratory (BBL)** that gives permission to take, possess, and transport feather samples (for how to get permission from the USGS BBL please see below). In addition, you will likely need permission from the **State agency** where you are collecting feathers. For more information about State specific requirements please visit: http://naturalhistory.si.edu/BIRDNET/permit/index.html. You **do not need** a USFWS scientific collecting permit to collect 2 body feathers from live birds while you are banding, **if you have permission from the Bird Banding Lab (http://www.fws.gov/forms/3-200-7.pdf)**

Copies of federal bird banding permits and state permits must be included in sample shipment

Collecting Feather Samples

To collect a sample, gently tease a part feathers on the belly/breast of the bird until you have a feathers firmly between your thumb and finger. To pluck the feathers, just hold them relatively close to the base, and pull gently. **Do not touch the quill**, as the DNA is extracted from the skin cells attached to it. Place the feathers, 2-3 total, from each bird into an envelope (if you need envelopes, please let us know!). Providing your own envelopes is fine, but please make sure each envelope contains the necessary data described below.

Feathers are preferably stored refrigerated at 4°C but can be stored at room temperature. Once feathers are refrigerated please keep them cool, and ship with ice packs. Please send sample shipments at the end of the field season (see instructions below).

Required Data

On each envelope please provide the following information:

The envelopes we are using are a part of the larger UCLA genoscape project that seeks to create genetically-based maps of 100 different bird species. Here we clarify the data requests on the envelope for the kestrel project.

- Date (Please use letters for the month instead of numbers, e.g. Apr 25, 2016)
- Species Name
- Band Number
- Location (closest town, State/Provence, country [eg: Boise, ID, USA])
- Sex (male or female)
- Age code: adult (A) or nestling (N) [if nestling please record age in days in the UKN line__]
- Please check yes for Breeding? If samples are from an adult bird captured on the nest or at a territory, otherwise check No or UKN.
- Recapture? If the bird was previously banded by you or another bander (yes) or the bird is newly banded (no)
- UCLA # _____ (leave blank)

Please include additional data on a **separate piece of paper** or email us a spreadsheet to fcphenology@boisestate.edu:

- **Date** (Please use letters for the month instead of numbers, e.g. Apr 25, 2016)
- **Band number**
- **GPS coordinates**: Please provide latitude and longitude in decimal degrees based on a WGS84 projection (to 6 decimal places, **e.g. 43.554987, -112.487325**)
- **State/Province**
- **Age**: We are interested in whether the kestrel is an adult (can fly) or nestling (in a nest) we are not distinguishing between hatch-year and after-hatch year birds. If the kestrel is a **nestling, please estimate the number of days old the nestling** is when it is sampled using the aging guide at the end of this document and record the age in days on the spreadsheet.
- **Sex**: male or female, please do not use symbols

Feel free to fill out the data sheet on page 5 this document for submitting your data with the envelopes or find an electronic version at https://fullcyclephenology.com/resources/

Shipping Feather Samples

For shipments **within the U.S**., please include the following:

1. A copy of the importers (UCLA's) USDA permit #48865 for shipments within the US [last 2 pages of this doc].
2. A copy of your (the collectors) federal bird banding permit and state permit (as necessary).
3. A datasheet with the above information
4. The samples

Returning Completed Samples to UCLA with necessary paperwork
Send your samples in a single shipment at the end of your field season using the provided return FedEx shipping labels (the address is also provided below). If shipping without the provided label please send via FedEx or UPS. **PLEASE** DO NOT USE THE U.S. postal service to send us your feather samples. The U.S. Postal Service irradiates mail with high-power radiation that might damage the DNA.

SHIP TO: Winnie Le, Center for Tropical Research, Institute of the Environment, University of California, Los Angeles, 610 Charles E. Young Drive East, 4162 TLSB, Los Angeles, CA 90095, USA; Tel: (310) 267-4460; E-mail: **lewinnie@ucla.edu**

Permits must be included in sample shipment

Thank you very much for your participation in this important work! Please let us know if you have any questions.

For additional information or questions, please contact:

Michaela S. Brinkmeyer or Anjolene Hunt
Boise State University
Tel: (208) 426-4923; e-mail: **fcphenology@boisestate.edu**
website: fullcyclephenology.com/

How to get permission to collect, possess, and transport American kestrel feather samples on a USGS BBL bird banding permit

(https://www.pwrc.usgs.gov/BBL/homepage/tissue_samp.cfm):

Tissue Sampling (feathers)

Please allow 2 months lead time to process your revision request.

To request permission to take feather samples, please provide the BBL the following information.

1. Master's name and permit number, and any subpermits that require this permission
2. Species
3. What feathers will be taken, and how many
4. Project proposal (Why are the feathers needed? How will they be used?)

This information can be sent to bbl_permits@usgs.gov.

Please feel free to use the following text for the permit applications.

Research Project Summary

Using high-resolution genetic markers to reveal American Kestrel connectivity: towards understanding kestrel population declines and the impacts of climate change on raptor monitoring and management

Summary

American Kestrels (*Falco sparverius*) are declining for unknown reasons and studies regarding their rates of survival and migratory ecology are lacking. Further, patterns of bird migrations are changing both spatially and temporally because of climate change. Historically, the challenge for studying migrant ecology, the drivers of regional population declines, and the impacts of climate change has been an inability to link breeding, wintering, and migrant populations. Recently, Ruegg et al (2014) described a new single nucleotide polymorphism (SNP) based method for identifying populations of migratory birds at finer spatial scales than has previously been possible. By harnessing the power of this new SNP-based methodology we will finally be able to tackle long-standing questions in migrant ecology and population dynamics of the American Kestrel. Understanding the migratory connectivity of populations of American Kestrels, and how connectivity changes with climate, will allow us to: 1) identify the wintering grounds and migratory routes of declining breeding populations,2) test hypotheses about population change and 3) assess the impacts of climate change on the effectiveness of migration counts as a tool for monitoring raptor populations. To do this, we will need to collect DNA samples using plucked feathers from American kestrels across their range and throughout the annual cycle. Two body feathers will be gently plucked from the belly or back of the kestrel, stored in an envelope, and shipped to UCLA for analysis.

Data sheet for feather samples used in the American Kestrel Genoscape Project

Example

Date	Band Number	Latitude	Longitude	State/Province	Country	Age	Nestling age (in days)	Sex	Bander’s Name
30-Jun-17	9876-54321	43.554987	-112.487325	Idaho	USA	Nestling	23	male	Julie Heath

U.S.DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
RIVERDALE, MARYLAND 20737
file:///D:/Inetpub/wwwroot/Epermits/Images/

UNITED STATES VETERINARY PERMIT FOR IMPORTATION AND TRANSPORTATION OF CONTROLLED MATERIALS AND ORGANISMS AND VECTORS

PERMIT NUMBER
48865
Research

DATE ISSUED
05/27/2016

DATE EXPIRES
05/27/2017

NAME AND ADDRESS OF SHIPPER(S)

Various shippers within the
UNITED STATES

CC:
Service Center, CA (Sacramento, CA)

NAME AND ADDRESS OF PERMITTEE INCLUDING ZIP CODE AND TELEPHONE NUMBER

Thomas B. Smith
University of California, Los Angeles
Dept. of Ecology and Evolutionary Biology
610 Charles E. Young Drive East
Los Angeles, California 90095-7239
310-206-6234 / 310-206-4712

U.S. PORT(S) OF ARRIVAL
TRANSPORT PERMIT

MODE OF TRANSPORTATION
ANY

AS REQUESTED IN YOUR APPLICATION, YOU ARE AUTHORIZED TO IMPORT OR TRANSPORT THE FOLLOWING MATERIALS

Avian blood, tissue, cloacal swabs and feather samples (previously imported)

RESTRICTIONS AND PRECAUTIONS FOR TRANSPORTING AND HANDLING MATERIALS AND ALL DERIVATIVES

THIS PERMIT IS ISSUED UNDER AUTHORITY CONTAINED IN 9 CFR CHAPTER 1, PARTS 94,95 AND 122. THE AUTHORIZED MATERIALS OR THEIR DERIVATIVES SHALL BE USED ONLY IN ACCORDANCE WITH THE RESTRICTIONS AND PRECAUTIONS SPECIFIED BELOW (ALTERATIONS OF RESTRICTIONS CAN BE MADE ONLY WHEN AUTHORIZED BY USDA, APHIS, VS).

- **Adequate safety precautions shall be maintained during shipment and handling to prevent dissemination of disease.**

- With the use of this permit I, Thomas Smith, Permittee, acknowledge that the regulated material(s) will be imported/transported within the United States in accordance with the terms and conditions as are specified in the permit. The Permittee is the legal importer/recipient [as applicable] of regulated article(s) and is responsible for complying with the permit conditions. The Permittee must be at least 18 years of age and have and maintain an address in the United States that is specified on the permit; or if another legal entity, maintain an address or business office in the United States with a designated individual for service of process; and serve as the contact for the purpose of communications associated with the import, transit, or transport of the regulated article(s). **Note: Import/Permit requirements are subject to change at any time during the duration of this permit.

- ***Each shipment shall be accompanied by an ORIGINAL signed document from the producer/manufacturer confirming that the transported material: 1) was derived only from avians (birds), and 2) was subjected to one of the following viral inactivation treatments prior to transport: a) heated to a minimum of 56°C for at least 3 hours, (b) heated to a minimum of 60°C for at least 30 minutes, ...[continued on page 2] ...

continued on subsequent page(s).....

TO EXPEDITE CLEARANCES AT THE PORT OF ENTRY, BILL OF LADING, AIRBILL OR OTHER DOCUMENTS ACCOMPANYING THE SHIPMENT SHALL BEAR THE PERMIT NUMBER

SIGNATURE	TITLE	NO. LABELS
Linda Kahn-Tobin Linda Kahn-Tobin	National Import Export Services	

RESTRICTIONS AND PRECAUTIONS: (continued from Permit Form VS 16-6)

- ... [continued from page 1] ... (c) heated to a minimum of 100°C for at least 20 minutes, ((d) treated with a minimum of 2% sodium dodecyl sulfate (SDS) for 30 minutes, (e) treated with a minimum of 3% beta propiolactone for 12 hours at 4°C at pH of 7, (f) immersed in a minimum of 10% formalin, (g) immersed in a minimum of 70% alcohol, (h) immersed in phenol/chloroform mixture, (i) treated with proteinase K, (j) treated with guanidine HCl, OR (k) placed on FTA cards (for liquids only) [This certification must CLEARLY correspond to the shipment by means of an invoice number or shipping marks or lot number or other identification method.]

- This permit DOES NOT authorize direct or indirect exposure of or inoculation into laboratory and domestic livestock, (including but not limited to: birds/poultry, cattle, sheep, goats, swine, and/or horses). Work shall be limited to *in vitro* uses only.

- Packaging, containers, and all equipment in contact with these materials shall be sterilized or considered a biohazard and be disposed of accordingly.

- This permit is valid only for work conducted or directed by you or your designee in your present U.S. facilities. (MATERIALS SHALL NOT BE MOVED TO ANOTHER U.S. LOCATION, OR DISTRIBUTED WITHIN THE U.S., WITHOUT USDA, APHIS, VS, NIES AUTHORIZATION.) ++EXCEPTION ++ Material is authorized to be distributed for evaluation, provided the transported material is accompanied by a signed document confirming that the material was subjected to one of the viral inactivation treatments specified above; Records of treatment and distribution shall remain on file and be made available to USDA upon request.

- Imported material may be subject to regulations enforced by the United States Department of Interior, Fish and Wildlife Service (FWS). Importer must contact FWS, information is available at web pages http://www.FWS.gov/permits/ and/or http://www.FWS.gov/le/travelers.html

- On completion of your work, all permitted materials and all derivatives therefrom shall be destroyed.

- The restrictions on this permit remain in force as long as the material is in the United States.

- This permit does not exempt the permittee from responsibility for compliance with any other applicable federal, state, or local laws and regulations.

- Any person who VIOLATES the terms and conditions of permits, and/or who forge, counterfeit, or deface permits may be subject to criminal and civil penalties in accordance with applicable law. In addition, all current permits may be cancelled and future permit applications denied.

U.S.DEPARTMENT OF AGRICULTURE
APHIS / VETERINARY SERVICES, RIVERDALE, MARYLAND 20737.

ATTACH TO U.S. VETERINARY PERMIT - 48865

RESTRICTIONS AND PRECAUTIONS: (continued from Permit Form VS 16-6)

oA copy of this permit must be included with the shipping documents.

Photoghraphic Guide
for Aging Nestling

AMERICAN KESTRELS

Gerald R. Griggs and Karen Steenhof
1993

U.S. Department of the Interior
Bureau of Land Management
Raptor Research and Technical Assistance Center
3948 Development Avenue
Boise, Idaho 83705

ACKNOWLEDGMENTS

In 1986 and 1987, nesting boxes were erected in Southwestern Idaho to study the food habits and breeding biology of American kestrels (*Falco sparverius).* The project was a cooperative effort between the Bureau of Land Management, the Idaho Department of Fish and Game, and the Idaho Transportation Department. Sixty-four nest boxes were placed on the backs of Interstate Highway 84 signs between Caldwell and Mountain Home, and in groves of trees near Kuna, Idaho. Personnel from the Raptor Research and Technical Assistance Center (RRTAC) and other investigators monitor the boxes annually to determine occupancy and nesting success and to band adults and nestlings to accumulate baseline data on dispersal and population dynamics.

Volunteers played a critical role in field work. Thanks to Dan Ambrose, Pam Dugger, Lauren Hemperley, Wes Schuessler, Sharon Stewart, Kurt Warmbier, and Becky Weeks for valuable assistance.

Thanks are also due to Michael Kochert, Bob Lehman, Leslie Carpenter, and George Carpenter for advice and review of the manuscript and to Kay Sundberg for typing the manuscript.

The general format and approach were adopted from aging guides by Marc Moritsch (1983a,b; 1985) for 3 other species of raptors.

INTRODUCTION

The purpose of this guide is to illustrate development stages of American kestrels (*Falco sparverius*) to help investigators time nest visits for banding, marking, and assessing nest productivity. This guide is based on observations of a single brood. Developmental rates vary with a number of factors such as sex, nutrition, position in brood, and brood size. Thus, ages assigned to chicks based solely on this guide should be used with caution.

METHODS

During routine monitoring of nest boxes, a clutch of American kestrel eggs was discovered in the midst of hatching on 17 June 1991. This nest box was on a highway sign about 24 km southeast of Boise. Two hatchlings (estimated at less than a day old), 1 pipped egg, and 2 intact eggs were present at that visit. The adult female was captured and banded. The box was next visited 7 days later (24 June), at which time there were 3 chicks and 1 intact egg. Beginning that day and every other day thereafter through 12 July, 35-mm photographs were taken of the 3 chicks in both black and white and color, and field notes were taken to document morphological development. Observations ended when young were 25 days old because they were within 1-3 days of flying. It should be noted that this brood hatched rather late in the nesting season. Of the 16 nest boxes that fledged young in 1991, only 2 hatched later than 17 June. The mean hatch date for those 16 broods was 20 May.

7 DAYS

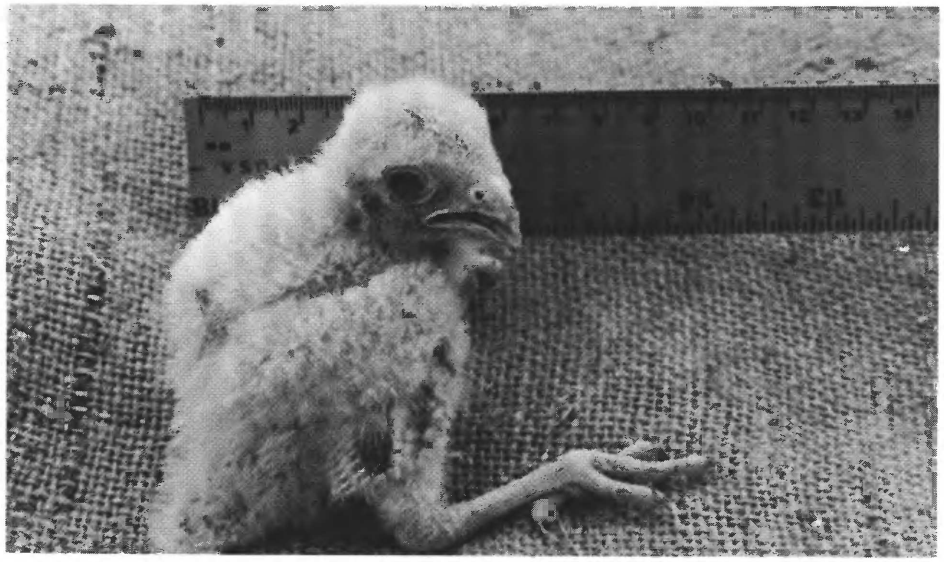
Remnants of egg tooth evident. Auriculars are downy white. Primary and greater primary covert sheaths are about 0.5 cm long; alula sheaths are slightly shorter. Rectrices and secondary sheaths, if present, are entirely obscured by down. A few scapular sheaths extend past down layer. In profile with wings folded, primary sheaths are not a prominent feature.

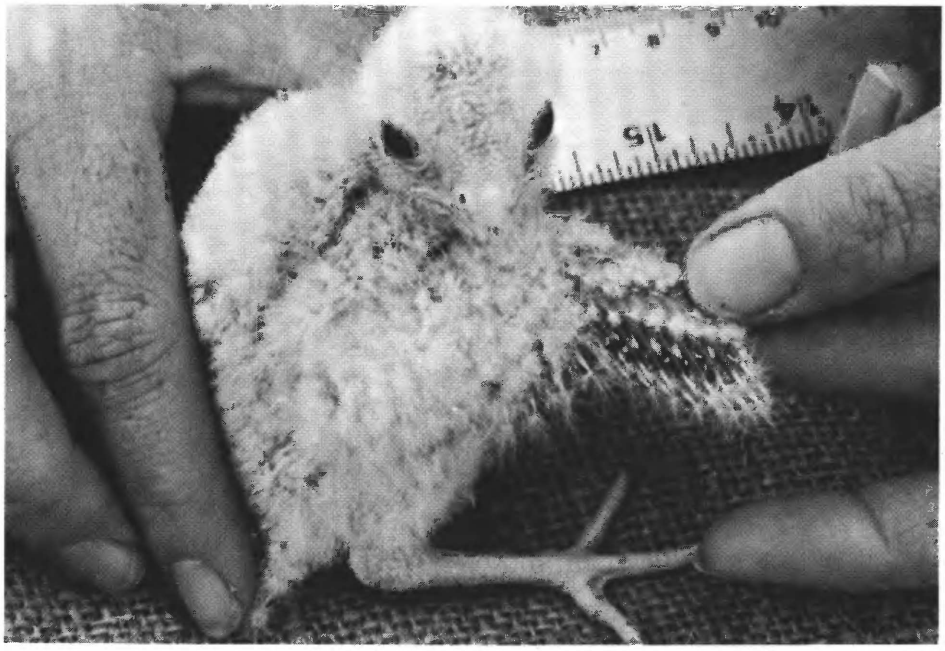
BEST INDICATOR: Intact primary sheaths approximately 0.5 cm long.

9 DAYS

Remnants of egg tooth barely discernable. Auriculars appear downy gray. With wings extended, all primary, greater primary covert, and alula sheaths are exposed and intact: primary and greater primary covert sheaths are about 1 cm long, alula sheaths about 0.5 cm long. A few secondary and rectrix sheaths and several scapular sheaths extend past the down layer. Feathers are beginning to emerge on the flank and upper breast showing a trace of brown. In profile, with wings folded, primary sheaths are a prominent feature.

BEST INDICATOR: Intact primary sheaths approximately 1 cm long.

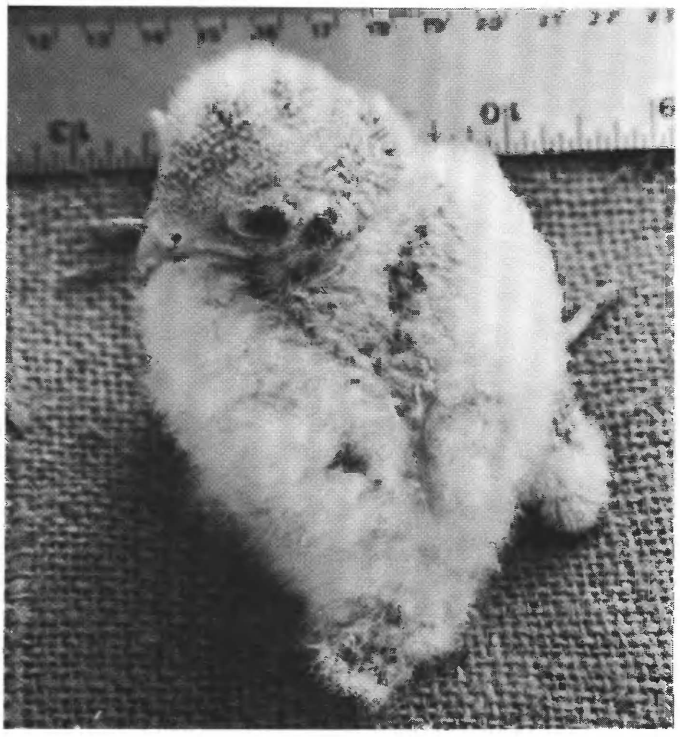




11 DAYS

No evidence of egg tooth. Auriculars appear as a dark gray patch. Occipitals show a trace of gray or brown color. With wings extended, all primary, greater primary covert, and alula sheaths are exposed and intact: primary and greater primary covert sheaths about 0.5 cm long, alula sheaths about 1 cm long. All secondary sheaths extend past the down layer; greater secondary coverts barely extend past the down layer. Intact rectrix sheaths extend about 0.5 cm beyond down. Rectrix coverts are beginning to emerge and show a trace of color. Scapular region is about 30% feathered; flank about 20% feathered; upper breast about 10% feathered. In profile with wings folded, primary sheaths are a dominant feature.

BEST INDICATOR: Intact primary sheaths approximately 1.5 cm long.



13 DAYS

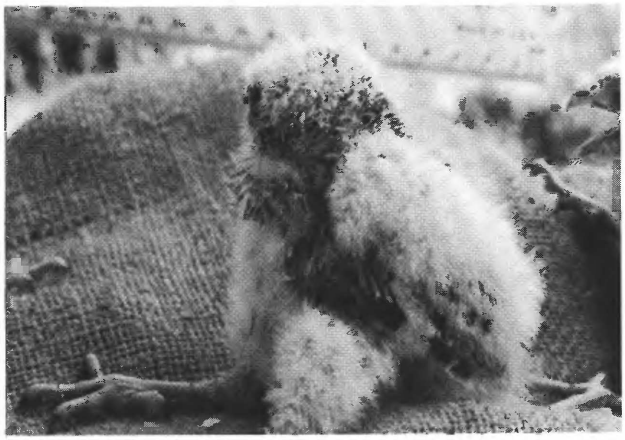
Remiges and rectrices have erupted from sheaths approximately 0.5 cm with neossoptile attached. Scapular region is about 40% feathered; flank about 30% feathered; upper breast about 10% feathered. The coronal region shows a trace of gray or brown and the occipital area is about 10% feathered. The auricular patch is about 80% black
feathers.

BEST INDICATOR: Flight feathers erupted from sheaths about 0.5 cm.

15 DAYS

Some flight feathers still have neossoptile attached. Remiges are unsheathed approximately 1 cm; rectrices and alulae are unsheathed about 0.75 cm. Scapulars are about 50% feathered and mostly obscure the back with wings folded. The flank is about 50% feathered; upper breast about 20% feathers. The occipitals are about 25% feathered, the crown shows only a trace of feathers, and the auricular patch is about 90% black feathers. In profile with wings folded, down over the lesser wing coverts form large, unbroken, white patches on each wing, surrounded on all margins by some colored feathers. In profile, these patches extend more than half the depth of the body. With wings folded and viewed from the back, these patches appear larger than the scapular feathered area.

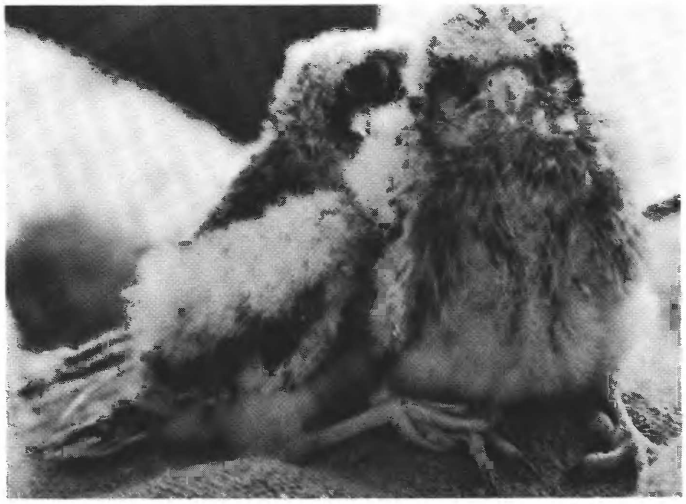
BEST INDICATORS: Remiges erupted from sheaths about 1 cm; in profile, solid down patches over lesser wing coverts are a dominant feature surrounded by some color.



17 DAYS

Primaries and greater primary coverts are unsheathed about 2.5 cm; secondaries, rectrices, and greater secondary coverts are unsheathed about 2 cm. Scapulars are about 95% feathered, flank about 75% feathered; breast about 50% feathered. The crown has a gray downy appearance, the occipitals about 40% feathered, and black auricular patches are fully feathered. With wings folded and viewed in profile, unbroken down patches over the lesser coverts are about half the depth of the body. With wings folded and viewed from any aspect, nestlings present about equal area of colored feathers and white down. At this stage of development, sex of young may be unambiguously determined from plumage.

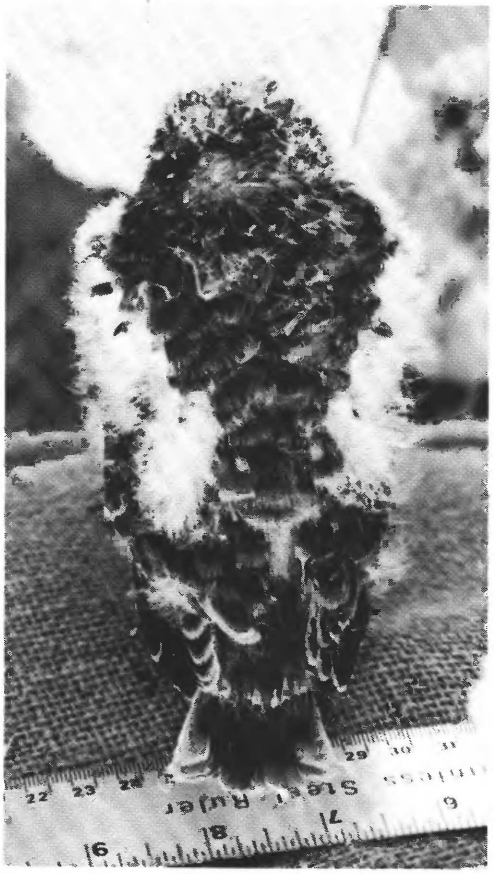
BEST INDICATORS: Remiges and rectrices erupted from sheaths 2 to 2.5 cm; overall, nestlings are about half feathers and half down.



19 DAYS

The breast is about 80% feathered: the characteristic streaked pattern in females and spotted pattern in males is evident. Primaries are unsheathed about 3 cm. In profile, down patches over lesser wing coverts are prominent features, but broken and less than a third of the body depth. The crown is about 50% feathered, forehead 30% feathered. Viewed from any aspect, nestlings show more color than down.

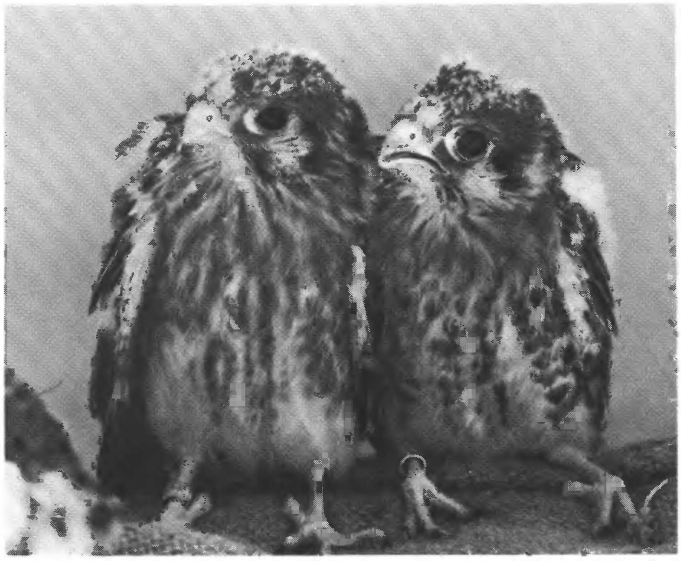
BEST INDICATORS: Primaries unsheathed about 3 cm. Crown about 50% feathered, forehead 30% feathered. Breast 80% feathered and shows streaks in females and spots in males. Down patches on lesser wing coverts are prominent, but broken and less than one-third of body depth.



21 DAYS

Breast is about 95% feathered. Primaries are unsheathed approximately 3.5 cm in males and about 4.5 cm in females. Down patches over lesser wing coverts are broken (about 30% feathered within the margin of the down patch) and less than one-third of the body depth. The coronal region is about 80% feathered, occipital plumage is about 95% feathered. Superciliary and auricular regions show only a trace of down.

BEST INDICATORS: Primaries unsheathed 3.5 - 4.5 cm. Crown about 80% feathered, occipital region about 95% feathered. Down patches over lesser wing coverts are broken (about 30% feathered) and less than one-third of the body depth.



23 DAYS

Fully feathered except for: 10% down on forehead, crown, back, and belly; 5% down on breast, legs, and the occipital area. Remnants of down patches over lesser secondary coverts may be present. Only traces of down elsewhere. Length of unsheathed portion of seventh primary: female, about 5.5 cm; male, about 4.5 cm. Rectrices: female about 3.5 - 4 cm; male about 2.5 - 3.5 cm.

BEST INDICATORS: Fully feathered except for 10% down on forehead, crown, back and belly, 5% down on breast, legs, and occipitals. Length of seventh primary: female about 5.5 cm, male about 4.5 cm.

25 DAYS

Fully feathered with only traces of down, principally on the crown and forehead.

GLOSSARY*

Alula - 3 or 4 feathers on the leading edge of the wing.

Auriculars - Feathers covering the ear opening and the area immediately around it.

Coverts - Collectively refers to all feathers of the wing and tail above the remiges and rectrices.

Coronals - Feathers on the crown (top of the head behind the eyes).

Flank - side of the body under and below the wing.

Forehead - Top of the head in front of the eyes.

Greater primary coverts - the first row of feathers covering the bases of the primaries.

Greater secondary coverts - the first row of feathers covering the bases of the secondaries.

Lesser wing coverts - For the purposes of this guide, includes all dorsal surface wing feathers above the greater primary and greater secondary coverts.

Occipitals - Feathers on the occiput (back of the head).

Primaries - Long feathers on the distal wing segment; numbered from innermost to outermost. Members of the order Falconiformes have 10 primaries.

Remiges - Collective term for primaries and secondaries.

Rectrices - Tail feathers. (Singular form is rectrix.)

Scapulars - A group of feathers on the shoulder, along the side of the back.

Secondaries - Long feathers on middle wing segment; numbered from outermost to innermost.

Sheath - keratin material which encases newly developed juvenile feathers. The sheath disintegrates and allows the feather to unfold.

Superciliary - the region immediately over the eye, between the eye and the crown.

Neossoptile - Natal down which sometimes remains attached to newly erupted juvenile feathers.

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*SOURCES: North American Bird Banding Manual (1977) and Pettingill (1970).

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Pettingill, Olin Sewall, Jr. 1970. Ornithology in Laboratory and Field, Fourth Edition. 524pp.

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