1 **Homing pigeons (*Columba livia*) modulate wingbeat characteristics as a function of**
2 **route familiarity**
3
4 Running title: Pigeon wingbeats and route learning
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15 Key words: airspeed, flight, navigation, route learning, wingbeat amplitude, wingbeat
16 frequency

17   **Summary statement**

18

19   Onboard accelerometry reveals that pigeons' flight characteristics undergo gradual changes
20   over the course of learning a route, and thus provide potential biomechanical signatures of
21   birds' landscape familiarity.

22

23   **Abstract**

24

25   Mechanisms of avian navigation have received considerable attention, but whether different
26   navigational strategies are accompanied by different flight characteristics is unknown.
27   Managing energy expenditure is critical for survival, therefore understanding how flight
28   characteristics, and hence energy allocation, potentially change with birds' familiarity with a
29   navigational task could provide key insights into the costs of orientation. We addressed this
30   question by examining changes in wingbeat characteristics and airspeed of homing pigeons
31   (*Columba livia*) as they learned a homing task. Twenty-one pigeons were released 20 times
32   individually from either 3.85 km or 7.06 km from home. Birds were equipped with 5 Hz GPS
33   trackers and 200 Hz tri-axial accelerometers. We found that, as the birds' route efficiency
34   increased during the first six releases, their median peak-to-peak dorsal body (DB)
35   acceleration and median DB amplitude also increased. This, in turn, led to higher airspeeds,
36   suggesting that birds fly slower when traversing unfamiliar terrain. By contrast, after route
37   efficiency stabilised, birds exhibited increasing wingbeat frequencies, which did not result in
38   further increases in speed. Overall, higher wind support was also associated with lower
39   wingbeat frequencies and with increased DB amplitude. Our study suggests that the cost of
40   early flights from an unfamiliar location may be higher than subsequent flights both due to
41   inefficient routes (increased distance) and lower airspeeds (increased time). Furthermore, the
42   results indicate, for the first time, that birds modulate their wingbeat characteristics as a
43   function of navigational knowledge, and suggest that flight characteristics may be used as
44   "signatures" of birds' route familiarity.

Introduction

Forward flapping flight is the most energetically demanding form of vertebrate locomotion (Norberg, 1990; Schmidt-Nielsen, 1972). To reduce the energetic cost of flight, birds utilise behavioural mechanisms, such as intermittent flight patterns (Rayner, 1985; Tobalske and Dial, 1996), formation flight (Weimerskirch et al., 2001), and modulation of their wingbeat frequency and amplitude (Greenewalt, 1962; Lilienthal, 2001). Whilst energy saving mechanisms have been identified, little is known about the extent to which birds employ these mechanisms in relation to navigation, for example, as a function of familiarity with a given landscape. Until now, avian navigation research has primarily focused on the sensory and cognitive underpinnings of orientation (Wiltschko and Wiltschko, 2009) and the total cost of a flight (Flack et al., 2016). However, a bird navigating from a familiar location, such as a roost or foraging site, may utilise different flight patterns compared to when flying over less familiar terrain where the distance to be covered and the route to be taken are less well known. Energy is frequently considered the currency of life (Butler et al., 2004), which means that managing energy expenditure is a key aspect of survival. Thus, energy allocation is likely to play a pivotal role in dictating the flight patterns utilised whilst navigating. Investigating whether birds modulate, for example, their wingbeat patterns in relation to their familiarity with a navigational task, could provide key insights into the cost of orientation.

Homing pigeons (*Columba livia*) are an ideal model species for studies investigating flight characteristics in relation to navigational knowledge due to their innate homing ability, amenability to experimental manipulation, and body size permitting the attachment of state-of-the-art tracking devices. For research subjects, this means we can ensure that the full navigational experience of a given individual is known and can be characterised over successive flights. Although the finer details are still debated, it is generally accepted that over unfamiliar terrain, pigeons navigate by utilising a combination of olfactory cues for position fixing (Gagliardo, 2013), and a solar and magnetic compass for directional guidance (Kramer, 1957; Schmidt-Koenig, 1990; Wiltschko and Wiltschko, 2005). In addition, over familiar terrain, pigeons are also able to utilise visual landmarks (Meade et al., 2005). Meade et al. (2005) found that homing pigeons released repeatedly from the same site developed stereotypical routes by the end of the experiment, with each individual having their own slightly indirect route. The results of this study and others indicate that pigeons reliably adopt such individually idiosyncratic routes as familiarity with the local landscape increases, and

79 rely more on visual landscape features as they become more experienced (Biro et al., 2004;

80 Guilford and Biro, 2014; Meade et al., 2005). Correspondingly, birds' route efficiency (the

81 straight line distance between the start and end of the route divided by the distance travelled

82 by the bird) increases steadily during the early stages of training and then plateaus once the

83 birds have developed stable idiosyncratic routes (Guilford and Biro, 2014; Meade et al.,

84 2005). Recapitulating a familiar but less direct route, rather than increasing route efficiency

85 further, suggests that there could be a higher energetic and/or cognitive cost associated with

86 navigating in an unfamiliar landscape for homing pigeons, relative to following a familiar

87 route. However, whether different navigational strategies during route learning are

88 accompanied by the same or different flight characteristics (e.g. wingbeat frequencies and

89 amplitudes) is still unknown.

90

91 Our study addresses this question by quantifying changes in the flight characteristics of

92 homing pigeons as they learn a homing task. Recent technological advances have led to the

93 introduction of miniature global positioning system (GPS) devices, which provide highly

94 accurate geographical position fixes over the full duration of a flight (Steiner et al., 2000),

95 and high-frequency tri-axial accelerometers, which can measure the acceleration of an animal

96 in three different planes, or dimensions, of movement (Halsey et al., 2009; Wilson et al.,

97 2006). In turn, the combination of GPS and accelerometers allows us to reconstruct birds'

98 routes, speeds, wingbeat frequencies and amplitudes of dorsal body (DB) displacement.

99 Although the precise relationship between the DB amplitude and the amplitude of wing

100 motions is unclear, the former can nevertheless be used as an indirect measure of the latter

101 (Hedrick et al., 2004; Usherwood et al., 2011). Together, wingbeat frequency and amplitude

102 are related to the variation in power and speed of flapping flight, and thus can be used as a

103 proxy for energy expenditure and/or work rate. By varying wingbeat frequency and

104 amplitude, a bird is able to adjust the costs of flight. Reducing wingbeat frequency reduces

105 the inertial power cost of the flight (i.e. power required to move the wings), as the power

106 requirement of horizontal steady flight is proportional to the square of wingbeat amplitude

107 but the cube of wingbeat frequency (Greenewalt, 1962; Lilienthal, 2001). By contrast,

108 increasing wingbeat frequency and decreasing amplitude reduces the drag of the wings and

109 body and increases lift, thereby optimising on aerodynamic efficiency (i.e., optimising the

110 forces acting on the bird relative to the air for efficient flight parameters) (Usherwood, 2009;

111 Usherwood et al., 2011). Thus, analysing variation in wingbeat characteristics and speed in

112 relation to navigational knowledge could shed new light on the costs of navigation and the
113 energetic strategies employed by birds as they learn a route home.
114
115 **Materials and methods**
116
117   *a) Subjects*
118
119 Twenty-one homing pigeons aged either 1 or 3 years were used. All birds had prior homing
120 experience, but had not been released at or near the sites used in the current study. The
121 subjects were housed with ~120 other pigeons in two neighbouring lofts at the Oxford
122 University Field Station, Wytham, UK (51°46'58.2"N, 1°19'2.7"W). Access to water, grit
123 and a standard pigeon feed mix were available *ad libitum* at all times in the loft. All subjects
124 were able to perform free flights around the loft on a daily basis throughout the year. In
125 addition, in the month immediately preceding the start of the experiment, all subjects
126 participated in a minimum of 24 solo or flock releases from four different release sites 1-3 km
127 to home, as basic homing training, to familiarise the birds with the catch and release
128 procedures and with being flown from an unfamiliar location. The protocols outlined in this
129 paper were approved by the Local Ethical Review Committee of the University of Oxford's
130 Department of Zoology.
131
132   *b) Experimental protocols*
133
134 Two release sites, both on a bearing of 282° from the loft, were selected. The "far" site
135 (Barnard Gate; 51°47'48.1" N, 1°25'3.3"W) was 7.06 km from the loft and the "near" site
136 (Mill Lane, Eynsham; 51°47'24.2"N, 1°22'19.5"W) was located 3.85 km from the loft.
137 Subjects were randomly assigned to two groups in an even age distribution. Group 1 (10
138 pigeons; 5 one-year-old and 5 three-year-old birds; mean ± s.d. body mass 471 ± 39 g) were
139 released 20 times individually from the "far" site, and Group 2 (11 pigeons; 6 one-year-old
140 and 5 three-year-old birds; 471 ± 20 g) were released 20 times individually from the "near"
141 site. Releases were conducted between May and July 2015, on days when the sun was visible
142 and the wind speed was < 7 m s^{-1} when measured 5.5 m above the ground. Subjects
143 participated in a maximum of two releases per day, with a minimum of three hours between
144 each release. All birds were released individually. Initially, releases occurred at 10-minute
145 intervals; this was later reduced to a minimum of 4 minutes if it could be visually confirmed

that the previously released bird had left the vicinity of the release site. If, at any point, a bird accidentally paired up with another subject, the release was excluded for both birds (18 flights). Four additional tracks were removed from the analysis. One bird failed to return home before the GPS battery ran out on its first release from the far site, two GPS faults occurred for birds during the fifth release and fourteenth release, and the devices could not be accurately synchronised for a bird on its tenth release. In addition, four birds landed during their first release. For these landings, the entire descent, stationary and ascent sections were removed from the track data from the point of first descent to the peak of ascent.

c) Data logging

The birds were tracked using 5 Hz GPS loggers (BT-Q1300ST, Qstarz International Co., Ltd., Taipei, Taiwan; 15 g) and 200 Hz tri-axial accelerometers (AX3, Axivity, Newcastle upon Tyne, UK; ± 16 g-force; 11 g). The loggers were attached to the pigeons using Velcro strips which were glued to trimmed feathers on the back (Fig. 1; Biro et al., 2002). In total, the loggers and fastenings weighed 27 g (less than 7 % of the subjects' mean body mass). Two weeks prior to the start of the experiment, clay weights (27 g) were attached to the birds' back via Velcro to accustom them to flying with the additional mass. These were exchanged for GPS devices and accelerometers immediately prior to each release. GPS and accelerometer data were downloaded using QTravel (Qstarz International Co., Ltd., Taipei, Taiwan; version 1.48(T)) and Open Movement (Om) GUI Application (Newcastle University; version 1.0.0.28), respectively.

The weather, including mean wind speed per minute and a running mean of the wind bearing over the previous ten minutes, was recorded using a WS2083 Professional Wireless Weather Station with USB upload (Aercus Instruments, Doncaster, UK) situated 5.5 m above the pigeon lofts. Weather data was logged using Cumulus Weather Station Software (Sandaysoft, Sanday, Orkney, UK; version 1.9.4).

d) Data processing

GPS and accelerometer data were synchronised to an accuracy of ± 0.2 s (GPS frequency) using the point of take-off in both the GPS and accelerometer loggers, which were identified using the marked increases in GPS speed and dorsal acceleration peaks produced during take-

off. The weather data was combined with the GPS and accelerometer data using the timestamps from the weather station and the GPS loggers. For each GPS point, the orthodromic (great-circular) distance travelled and birds' final bearing from the previous point were calculated using the haversine formula and forward azimuth, respectively. For each flight, route efficiency was calculated as the ratio between the total straight-line (great-circular) distance between release and home, divided by the sum of the direct (great-circular) distances between each successive GPS point (straightness index; Batschelet, 1981). Wind support and crosswind were calculated using the methods described in Safi et al. (2013): wind support represents the length of the wind vector in the direction of the birds' flight and crosswind represents the absolute speed of the wind vector perpendicular to the birds' direction of travel (Fig. S1). Airspeed, the speed of the bird relative to the wind, was then calculated using the speed derived from the GPS devices while taking into account wind support and crosswind (Safi et al., 2013).

The dorsal (Z-axis) accelerometer measurements were filtered by taking a running mean over five data points (0.025 s). Static acceleration (or gravity) was removed by subtracting a running mean over 15 wingbeat cycles (> 2 s). The running mean was calculated over wingbeat cycles rather than over specific time periods, because variation in wingbeat frequencies would have meant including varying quantities of partial wingbeat cycles in a time-based running mean. The dorsal acceleration signal was then used to detect each wingbeat using the upper reversal point in acceleration (Fig. S2; Norberg, 1990; Portugal et al., 2014). The peak-to-peak dorsal body (DB) acceleration (g) and wingbeat frequency (number of wingbeats per second; Hz) were calculated for each individual wingbeat. The amplitude of the DB displacement (mm), which is the amount the body is displaced per wingbeat, was then calculated by the double integration of dorsal accelerometer measurements (Usherwood et al., 2011). After the first integration running mean over 15 wingbeat cycles removed from velocity to remove drift and then the data was filtered using a fourth order high-pass Butterworth filter with a cut-off frequency of 1 Hz. The procedure was repeated after the integration for displacement but with a cut-off frequency of 2.5 Hz in the Butterworth filter. The cut-off frequencies were determined by visualising the data using fast Fourier transforms. A more conservative estimate of the amplitude of the DB displacement was also calculated by passing the raw accelerometer measurements through fourth order Butterworth filters prior to integration instead of running means, but this led to no significant difference in the results (Fig. S3).

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In order to only compare sections of steady flight, the data were trimmed in a 1000 m radius around the release site (start point) and the pigeon lofts (end point). The shortest straight-line distance of the entire steady flight therefore measured 5.08 km for the far site and 1.85 km for the near site, respectively. In addition to comparing the entire steady flight, the data were also trimmed to remove sections with lower wingbeat frequencies ($\leq$ 3.0 Hz) and sections of tortuous flight to remove any effect of gliding, idling or circling from the dataset. Tortuosity was calculated by taking a running mean of the change in the birds' bearing over every one second of data (5 GPS points), with changes in direction of $\geq 3.0°$ removed in order to discard circling and keep only active straight-line powered flight.

e) Data analyses

Piecewise linear mixed effects (LME) models were used investigate the effect of repeated releases on route efficiency, median peak-to-peak DB acceleration per wingbeat (g), median DB amplitude per wingbeat (mm), median wingbeat frequency (Hz) and median airspeed (m s^{-1}). Piecewise, or segmented, regression identifies an abrupt change of the dependent variable (or breakpoint) in respect to the independent variable, which allows one to fit pre- and post-event slopes (Naumova et al., 2001). We used this approach to identify if changes in wingbeat characteristics corresponded to changes in route efficiency and to identify the trends in the data either side of this change. Breakpoints were objectively estimated using one-dimensional optimisation. Piecewise LME models were fitted using the fixed-effects of release number less than the breakpoint (breakpoint − release number), release number greater than the breakpoint (release number − breakpoint), median wind support, median crosswind and group. Individual was also added as a random slope on both release number effects.

To establish the effect of wingbeat characteristics on airspeed, two LME models were used. Firstly, with median peak-to-peak DB acceleration and median wingbeat frequency as fixed-effects, and secondly with median DB amplitude per wingbeat, which is dependent on peak-to-peak DB acceleration (force exerted on the dorsal body) and wingbeat frequency (duration of the wingbeat), as a fixed-effect. Group was added as a fixed-effect to both models but was insignificant. Individual was added as a random-effect on the intercept. In addition, LME models were used to directly relate route efficiency with wingbeat characteristics and

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248   airspeed between releases 1-6 using the fixed-effects of route efficiency, median wind

249   support, median crosswind and group, and the random-effect of individual.

250

251   Route efficiency was negatively skewed, and was therefore transformed before analysis by

252   directly inverting values and taking the logarithm using the formula $log10(1 - x)$. From the

253   full models, simpler models were obtained by stepwise deletion of non-significant terms.

254   Likelihood ratio tests were used to test the statistical significance of each fixed effect in the

255   best-fitting model. LME models were calculated using maximum likelihood and the models

256   were checked for assumptions of linearity, normality, homoskedasticity and autocorrelation

257   by visual inspection of plotted residuals. Model fit was assessed by calculating conditional R-

258   squared values ($R^2_{LME(C)}$) using the methods described in Nakagawa and Schielzeth (2013).

259

260   Data processing and analysis were conducted using MATLAB (MathWorks, Natick, USA;

261   version R2015a) and the open-source software R (R Core Team, 2016; version 3.2.3) using

262   the packages lme4 (Bates et al., 2015; version 1.1-8) and MuMIn (Bartoń, 2015; version

263   1.15.1).

264

265   **Results**

266

267   Route efficiency improved as the birds became more experienced (Fig. 2). Over the first five

268   releases, route efficiency increased significantly from 0.46 ± 0.27 (mean ± s.d.) for release 1

269   to 0.82 ± 0.18 for release 5 (piecewise linear mixed model parameter estimate = 0.287;

270   likelihood ratio test for the model without release number: χ^2_1 = 32.9, P <0.001). A

271   breakpoint, which denotes a change of the dependent variable (route efficiency) in respect to

272   the independent variable (release number), was then automatically detected between releases

273   5 and 6 (5.5, 95% confidence interval (CI) [4.3, 6.2]; Fig. 3). No significant difference in

274   route efficiency was detected among releases ranging from release 6 (0.88 ± 0.10) to release

275   20 (0.87 ± 0.10; χ^2_1 = 0.7, P = 0.390). Group (i.e. release distance) also had a significant

276   effect on route efficiency, with birds flying from the nearer site (Group 2) flying significantly

277   more efficient routes than those released at the far site (Group 1; estimate = 0.318, χ^2_1 = 4.8,

278   P = 0.029). However, group had no significant effect on wingbeat characteristics or speed,

279   thus the results from the two groups were pooled for the remainder of the analyses (Table 1).

280   Route efficiency was also significantly affected by both median wind support (estimate = -

281 0.05, $\chi_1^2 = 14.8$, $P < 0.001$) and median crosswind (estimate = 0.10, $\chi_1^2 = 7.9$, $P = 0.005$), with

282 greater wind support and lower crosswinds associated with higher route efficiency.

283

284 A breakpoint was detected in the median peak-to-peak DB acceleration (5.6, 95% CI [4.1,

285 6.8]) and in the median DB amplitude (5.99, 95% CI [4.6, 6.9]) between the same release

286 numbers (5 and 6) as route efficiency (5.5, 95% CI [4.3, 6.2]). Both the DB acceleration and

287 amplitude significantly increased prior to the breakpoint (acceleration: estimate = 0.038, $\chi_1^2 =$

288 7.4, $P = 0.007$; amplitude: estimate = 0.140, $\chi_1^2 = 7.3$, $P = 0.007$) before decreasing

289 (acceleration: estimate = -0.006, $\chi_1^2 = 7.2$, $P = 0.007$; amplitude: estimate = -0.067, $\chi_1^2 =$

290 17.2, $P < 0.001$; Fig. 4). By contrast, the breakpoint for median wingbeat frequency appeared

291 later, between releases 9 and 10 (9.6, 95% CI [4.5, 12.6]), with no significant change prior to

292 the breakpoint ($\chi_1^2 = 2.0$, $P = 0.159$). Upon visual inspection one might argue that there is a

293 breakpoint in the median wingbeat frequency around release 6. Indeed, manually moving the

294 breakpoint to the breakpoints of DB acceleration (5.6) and amplitude (5.99) revealed that

295 wingbeat frequency significantly decreased during the early releases (breakpoint 5.6: estimate

296 = -0.03, $\chi_1^2 = 2.83$, $P = 0.007$; breakpoint 5.99: estimate = -0.02, $\chi_1^2 = 6.89$, $P = 0.009$).

297 However, the resultant models were weaker and accounted for less of the variability, which is

298 why the objective breakpoint is situated slightly later between releases 9 and 10. The visual

299 ambiguity in the breakpoint is reflected in the confidence interval, which is large for median

300 wingbeat frequency (95% CI [4.5, 12.6]). After the objective breakpoint, wingbeat frequency

301 increased significantly (estimate = 0.02, $\chi_1^2 = 18.6$, $P < 0.001$).

302

303 Median wind support also had a significant effect on both median wingbeat frequency and

304 median DB amplitude, with higher wind support associated with lower wingbeat frequencies

305 (estimate = -0.02, $\chi_1^2 = 26.5$, $P < 0.001$) and increased DB amplitude (estimate = 0.20, $\chi_1^2 =$

306 25.3, $P < 0.001$). By contrast, median wind support had no effect on peak-to-peak DB

307 acceleration, and median crosswind had no effect on any of the wingbeat characteristics.

308

309 Median airspeed (m s^{-1}) increased during the first five releases (breakpoint 5.2, 95% CI [3.9,

310 7.8]; estimate = 0.53, $\chi_1^2 = 32.5$, $P < 0.001$) and thereafter decreased slightly (estimate = -

311 0.07, $\chi_1^2 = 10.6$, $P < 0.001$). The mean of the median flight airspeeds for the first three

312 releases were particularly low (release 1: 20.1 ± 1.6 m s^{-1}; release 2: 19.2 ± 1.4 m s^{-1}; release

313 3: 19.7 ± 1.0 m s^{-1}). However, the coefficient of determination for the relationship between

median airspeed and release number was low, even when accounting for individual variation ($R^2_{LME(C)}$ = 0.22). By analysing all 20 releases in a LME model with median peak-to-peak acceleration and median wingbeat frequency as fixed-effects, we found that higher airspeeds were associated with higher peak-to-peak DB accelerations (estimate = 1.57, χ^2_1 = 10.3, P = 0.001) and lower wingbeat frequencies (estimate = -0.78, χ^2_1 = 3.9, P = 0.048). Furthermore, in a model with DB amplitude (displacement) as a fixed-effect, which is dependent on peak-to-peak DB acceleration (force exerted on the DB) and wingbeat frequency (duration of the wingbeat), DB amplitude was positively associated with airspeed (estimate = -0.24, χ^2_1 = 23.3, $P < 0.001$).

The results thus far indicate that changes in wingbeat characteristics and airspeed correspond to changes in our route familiarity variable (i.e., route efficiency). To directly relate these findings, LME models were used with route efficiency as a fixed-effect between releases 1-6. The results of these analyses corroborate these findings with median peak-to-peak DB acceleration (estimate = -0.11, χ^2_1 = 32.5, $P < 0.001$), median DB amplitude per wingbeat (estimate = -0.86, χ^2_1 = 20.9, $P < 0.001$) and median airspeed (estimate = -0.84, χ^2_1 = 20.2, $P < 0.001$) all positively related to route efficiency (negatively related to the transformed route efficiency; Table S1; Fig. S4). As with release number, median peak-to-peak acceleration was only influenced by route efficiency, with no significant effect of median wind support, median crosswind or group ($P > 0.1$). By contrast to DB acceleration, DB amplitude and airspeed, higher median wingbeat frequencies were associated with lower route efficiencies in releases 1-6 (estimate = 0.06, χ^2_1 = 8.1, P = 0.004). Although there was a significant difference between groups in route efficiency in the piecewise LME model (estimate = 0.318, χ^2_1 = 4.8, P = 0.029), there was no significant difference between groups when relating wingbeat characteristics and airspeed to route efficiency ($P > 0.1$).

We hypothesised that one potential explanation for differences in wingbeat characteristics could be changes in wingbeat modes with navigational experience, such as additional circling behaviour and gliding in the early releases. Analysing only data with flap frequencies > 3 Hz and tortuosity < 3° (i.e. without gliding and circling segments, respectively) resulted in a shift in the breakpoint in wingbeat frequency from release 9.6 (95% CI [4.5, 12.6]) to release 6.2 (95% CI [4.6, 12.5]). In addition, the wingbeat frequencies significantly decreased prior to the breakpoint (estimate = 0.02, χ^2_1 = 4.6, P = 0.03). However, aside from the slight changes

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347   to median wingbeat frequency, removing circling and gliding had no other significant effect

348   on the results other than decreasing median wingbeat frequency and median peak-to-peak DB

349   acceleration, and increasing median DB amplitude and median airspeed (Fig. S5).

350   Furthermore, it is important to note that the confidence interval in the breakpoint for

351   wingbeat frequency remains approximately the same and that on visual inspection one could

352   place a breakpoint in the whole flight data at around release 6, as discussed above.

353

354   **Discussion**

355

356   We investigated the effect of navigational experience in relation to wingbeat characteristics

357   and airspeed in homing pigeons. The results indicate, for the first time, that gradual increases

358   in the birds' route efficiency, observed as birds become more experienced with a given

359   terrain, are also accompanied by changes in wingbeat characteristics and airspeed. As the

360   birds' route efficiency improved during the first six releases, the median peak-to-peak dorsal

361   body (DB) acceleration, the median DB amplitude and, consequently, the birds' median

362   airspeed also increased. By contrast, after route efficiency stabilised, the birds' median DB

363   acceleration and amplitude decreased, whereas median wingbeat frequency increased.

364   However, decreasing DB amplitude, and hence wingbeat amplitude, in favour of increasing

365   wingbeat frequency did not result in a higher airspeed. Taken together, our results suggest

366   that birds may be modulating their flight characteristics as a function of navigational

367   familiarity with the area through which they are travelling.

368

369   As with previous studies (reviewed in Guilford and Biro, 2014), route efficiency improved

370   over consecutive releases. It increased significantly over the first five releases, with a

371   breakpoint detected between releases 5 and 6. The breakpoint denotes a change in route

372   efficiency (dependent variable) in respect to release number (independent variable), with the

373   position of the breakpoint determined by where the slopes of the two segments join. We

374   chose the piecewise linear mixed model approach to objectively identify a change of state

375   because, as these results demonstrate, wingbeat characteristics and airspeed continue to

376   change long after route efficiency stabilises. Whilst we could manually divide the data into

377   segments, an automated approach enables us to objectively identify changes in wingbeat

378   characteristics and airspeed as a function of release number and to identify whether these

379   changes correspond to changes in route efficiency. In this study, we identified that route

380   efficiency increases up to a breakpoint of 5.5 and thereafter stabilises. As expected, the near-

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381   site group (straight-line distance: 3.85 km) flew significantly more efficient routes home in

382   the early stages of route learning than the far-site group (7.06 km), due to the proximity of the

383   near release site to the familiar area surrounding the pigeons' home lofts. However, despite

384   these differences, no significant difference was found between groups in wingbeat

385   characteristics and speed, and route efficiency still significantly increased over the first five

386   releases for both groups. These results reinforce the idea that birds modulate their wingbeat

387   characteristics in response to navigating an unfamiliar route, and that this effect is detectable

388   even at short distances from home.

389

390   The aforementioned changes in route efficiency did correspond to changes in wingbeat

391   characteristics, both in terms of the positioning of the breakpoint and when directly relating

392   these factors during the first six releases. As route efficiency increased, the peak-to-peak DB

393   acceleration and DB amplitude also increased, which indicates the acceleration, or force, the

394   dorsal body experienced increased over the first few releases. The median peak-to-peak DB

395   acceleration per wingbeat was especially low during the first few releases. Although DB

396   acceleration did decrease again after route efficiency stabilised, the DB accelerations the

397   birds experienced during the first few releases were still much lower than in the last few

398   releases, which could suggest that the overall flapping force was lower during the first few

399   releases. Correspondingly, the airspeeds of the first three flights were also particularly low.

400   By analysing the influence of peak-to-peak DB acceleration and wingbeat frequency on

401   airspeed, we established that higher airspeeds were associated with higher peak-to-peak

402   accelerations and lower wingbeat frequencies, with peak-to-peak acceleration having a

403   slightly larger effect than wingbeat frequency. However, the amplitude of the DB

404   displacement, which is dependent on the peak-to-peak acceleration (force exerted on the DB)

405   and the wingbeat frequency (duration of the wingbeat), had the greatest overall effect on

406   airspeed. For example, a wingbeat which is both high in force (peak-to-peak acceleration)

407   and long in time (low wingbeat frequency) will result in a greater displacement, and hence

408   higher airspeed, than one that is low in force or short in time. It should be noted, however,

409   that this negative relationship between wingbeat frequency and airspeed is within the

410   subtleties of active flight parameters, and may not represent the relationship over the entire

411   range of the pigeons' wingbeat characteristics. Although DB amplitude is an indirect measure

412   of wing amplitude, the two measures are likely to be related (Hedrick et al., 2004). An

413   alternative explanation for the changes in wingbeat characteristics and airspeed could be

414   changes in flight behaviour, such as increased circling and/or gliding. However, removing

both wingbeat frequencies below 3 Hz and tortuous flight made no substantial difference to the results other than shifting the breakpoint of median wingbeat frequency, suggesting that the changes in wingbeat characteristics are occurring during straight-line powered flight. Flying at a slower speed from an unfamiliar location may be an advantage as it may enable the bird to gather more local ambient information.

Breakpoints in the median peak-to-peak acceleration (release: 5.7) and the median DB amplitude (5.99), which occur between the same release number as route efficiency (5.5), along with the fact that higher peak-to-peak acceleration and DB amplitude were associated with higher route efficiency in releases 1-6, indicate that DB movements change as a function of navigational knowledge. By contrast, during the first six releases, higher wingbeat frequencies were associated with lower route efficiencies. The large confidence interval and slightly later breakpoint in wingbeat frequency (9.6) may reflect the fact that it is likely that birds continue to learn routes home even after route efficiency initially plateaus. Indeed, removing circling and gliding behaviour resulted in a breakpoint in wingbeat frequencies being detected at release 6.2. Thus, changes in wingbeat characteristics shortly after the route efficiency breakpoint could still be related to the acquisition of navigational knowledge. An alternative explanation could be increases in the birds' physical fitness, or acclimatisation to the sensor mass or to the capture and release procedure. However, given the substantial number of releases immediately prior to the start of the experiment (≥ 24), the daily free flights around the loft and that the birds were fitted with clay weights equal to the size and mass of the devices two weeks prior to the start of the experiment, this is unlikely to have been a factor. Furthermore, migratory species, such as barnacle geese (*Branta leucopsis*) undertaking 2,500 km journeys, do not increase flight behaviour prior to migratory departure (Portugal et al., 2012), suggesting exercise is not a prerequisite for extended flight.

Shortly after route efficiency stabilised, changes in the birds' flight characteristics consisted largely of increases in wingbeat frequency as the birds' DB acceleration and amplitude decreased. The inertial power requirement, or cost, of horizontal steady flight is proportional to the square of wingbeat amplitude but the cube of wingbeat frequency (Greenewalt, 1962; Lilienthal, 2001), which means increasing wingbeat frequency would result in a higher inertial power cost. If this higher wingbeat frequency translated into higher airspeeds then this strategy could be beneficial as the bird would then reach its destination in less time (Hedenström and Alerstam, 1995). However, as already highlighted, higher wingbeat

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frequencies were associated with lower airspeeds. Indeed, airspeed did decrease significantly from release 6, although variability in this was high and the effect size small. Albeit increasing wingbeat frequency would increase the inertial power costs, a higher wingbeat frequency would also result in a higher aerodynamic efficiency by reducing the drag from the wings and body and increasing lift (Greenewalt, 1962; Lilienthal, 2001), which may be advantageous once the total length of the journey is known. The median wingbeat frequency increased in this study from 4.9 (release 6) to 5.2 (release 18), i.e. by 5.8%. However, these wingbeat frequencies are still considerably lower than those reported for homing pigeons participating in flight which may require a higher degree of aerodynamic efficiency or stability, such as flock flight (~6.6-7.0 Hz; Usherwood et al., 2011) and during ascending and descending flight (6.1-9.6 Hz; Berg and Biewener, 2008). Thus, the increases in wingbeat frequency exhibited in this study are more likely to be related to birds optimising their flight patterns between inertial power requirements and aerodynamic efficiency.

Increasing aerodynamic efficiency, by increasing wingbeat frequency, could help conserve energy particularly as the results of this study also indicate a strong effect of the wind on route efficiency and flight characteristics. The effect of wind on flight is well documented, particularly in relation to the timing and distance travelled during migration (Alerstam, 1979; Liechti and Bruderer, 1998). Liechti (2006) highlighted that wind speed can easily double or halve the bird's speed and thereby affect the overall cost of the flight. Weather data used in this study (mean wind speed and bearing) were recorded 5.5 m above the pigeon lofts, 7.06 km from the "far" release site. Therefore, the accuracy of the readings decreased the further away the bird was from the lofts, which could explain some of the variation, particularly in airspeed, found in this study. Nevertheless, the results indicate that wind support, in particular, was a consistent and significant factor contributing to the work rate, with a higher wind support (tail wind) associated with a lower wingbeat frequency, which would decrease the inertial power costs. By contrast, the birds' peak-to-peak DB acceleration was not affected by the wind, which suggests that, in winds under 7 ms^{-1}, birds compensate for the wind by modulating wingbeat frequency rather than the amplitude. Indeed, the change in breakpoint in the wingbeat frequency from the whole flight data to the active straight-line data from release 9.6 to release 6.2 could also be a reflection of the birds utilising circling and gliding behaviour to compensate for the effects of the wind. Furthermore, higher route efficiency was associated with higher wind support and lower crosswinds, which suggests

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482 that the birds may modify their route (and hence their route efficiency) depending on the
483 wind conditions.

484

485 Regardless of the underlying cause for the changes in wingbeat characteristics, the results of
486 this study indicate, for the first time, that pigeons modulate their wingbeat characteristics as a
487 function of navigational knowledge, which suggests that navigation and the learning process
488 in birds may have physical manifestations. In particular, the birds' peak-to-peak DB
489 acceleration was especially low during the first few releases, which was unaffected by wind
490 characteristics, and related to a lower airspeed. Thus, measuring flight characteristics could
491 provide new insights into the cognitive state of the bird. For example, wingbeat
492 characteristics may be used as "signatures" of birds' route familiarity, which could be utilised
493 in navigation research to identify how familiar a bird is with a given navigational task.
494 Furthermore, the results of this study lead us to speculate that birds may be able to orient and
495 learn more effectively at lower speeds, in which case the flight would then become a trade-
496 off, or compromise, between optimal navigation and learning (accuracy), and vulnerability to
497 predators and the total flight time (speed). Moreover, these results suggest that the cost of the
498 first flight, such as the first migration or first route out in search of a novel foraging site, may
499 be higher than that of subsequent flights, both due to inefficiencies in the route, which
500 increase the total distance flown, and the increased flight time, due to lower airspeeds. This in
501 turn could impact on the total time available for foraging and reproduction, and could impact
502 on other decisions, such as whether to fly with other individuals. For example, despite the
503 additional energetic cost of flying in a close cluster flock (Usherwood et al., 2011), a naïve
504 individual may be able to conserve energy by flying with experienced individuals to reduce
505 the total flight time, as well as gaining protection from predators. Thus, studying intra-
506 individual modulations of wingbeat characteristics and airspeed could provide new insights
507 into the decision-making and navigational strategies of birds.

508

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532

533   Fig. 1. A homing pigeon with an Axivity AX3 accelerometer (Newcastle upon Tyne, UK;
534   front) and QStarz BT-Q1300ST GPS logger (Qstarz International Co., Ltd., Taipei, Taiwan;
535   case removed; back) attached to the back via Velcro strip, which was glued to trimmed
536   feathers. Scale bar shows 4 cm.

537

538   Fig. 2. Examples of routes flown during the first (red; release 1) and last release (blue; release
539   20) from (A) the far site and (B) the near site. Note the increase in route efficiency at both
540   sites. Map designed using ArcGIS 10.4.1 (Esri Inc., Redlands, USA) using the World
541   Topographic Map (Esri et al., 2013). Scale bar shows 3 km.

542

543   Fig. 3. Route efficiency (mean ± s.e.m.) as a function of release number for Group 1 (far site,
544   n = 10) and Group 2 (near site, n = 11). The dashed line indicates a computationally
545   optimised piecewise linear mixed model breakpoint (± 95% C.I. indicated by dotted lines)
546   denoting a change in response function in respect to release number. Solid lines correspond to
547   local polynomial regression fitting.

548

549   Fig. 4. (A-C) Wingbeat characteristics and (D) airspeed (mean ± s.e.m., n =21) as a function
550   of release number. Dashed lines indicate computationally optimised piecewise linear mixed
551   model breakpoints (± 95% C.I. indicated by dotted lines) denoting a change in response
552   function in respect to release number. Solid lines correspond to local polynomial regression
553   fitting.

554

555   Table 1. Comparison of the piecewise linear mixed effects models for efficiency, wingbeat
556   characteristics and airspeed for releases 1-20. The fixed-effects with P-values and parameter
557   estimates (Est.) denoted in **bold** are included in the final model. Conditional R-squared values
558   ($R^2_{LME(C)}$) are calculated using the methods described in Nakagawa and Schielzeth (2013).

559

560

Table 1.

	Breakpoint (95% CI)	Release number < breakpoint		Release number > breakpoint		Wind support		Crosswind		Group		$R^2_{\text{LME(C)}}$
		P-value	*Est.*	*P-value*	*Est.*	*P-value*	*Est.*	*P-value*	*Est.*	*P-value*	*Est.*	
Efficiency (transformed)	5.5 (4.3, 6.2)	**<0.001**	**0.287**	0.390	-	**<0.001**	**-0.049**	**<0.001**	**0.099**	**0.029**	**0.316**	0.69
Median peak-to-peak DB acceleration (g)	5.6 (4.1, 6.8)	**0.007**	**0.038**	**0.007**	**-0.006**	0.487	-	0.679	-	0.286	-	0.65
Median DB amplitude per wingbeat (mm)	5.99 (4.6, 6.9)	**0.004**	**0.294**	**<0.001**	**-0.123**	**<0.001**	**0.195**	0.430	-	0.288	-	0.70
Median wingbeat frequency (Hz)*	9.6 (4.5, 12.6)	0.159	-	**<0.001**	**0.016**	**<0.001**	**-0.025**	0.302	-	0.735	-	0.68
Median airspeed (m s^{-1})	5.2 (3.9, 7.8)	**<0.001**	**0.527**	**0.001**	**-0.065**	N/A	-	N/A	-	0.281	-	0.22

* Note: manually moving the breakpoint for wingbeat frequency revealed a significant decrease during the early releases (breakpoint 5.6: estimate = -0.03, χ^2_1 = 2.83, P = 0.007; breakpoint 6.0: estimate = -0.002, χ^2_1 = 6.88, P = 0.009)