RESEARCH ARTICLE

Flying high: limits to flight performance by sparrows on the Qinghai-Tibet Plateau

Yan-Feng Sun[1,2], Zhi-Peng Ren[1], Yue-Feng Wu[1], Fu-Min Lei[3], Robert Dudley[4,*] and Dong-Ming Li[1,*]

ABSTRACT

Limits to flight performance at high altitude potentially reflect variable constraints deriving from the simultaneous challenges of hypobaric, hypodense and cold air. Differences in flight-related morphology and maximum lifting capacity have been well characterized for different hummingbird species across elevational gradients, but relevant within-species variation has not yet been identified in any bird species. Here we evaluate load-lifting capacity for Eurasian tree sparrow (*Passer montanus*) populations at three different elevations in China, and correlate maximum lifted loads with relevant anatomical features including wing shape, wing size, and heart and lung masses. Sparrows were heavier and possessed more rounded and longer wings at higher elevations; relative heart and lung masses were also greater with altitude, although relative flight muscle mass remained constant. By contrast, maximum lifting capacity relative to body weight declined over the same elevational range, while the effective wing loading in flight (i.e. the ratio of body weight and maximum lifted weight to total wing area) remained constant, suggesting aerodynamic constraints on performance in parallel with enhanced heart and lung masses to offset hypoxic challenge. Mechanical limits to take-off performance may thus be exacerbated at higher elevations, which may in turn result in behavioral differences in escape responses among populations.

KEY WORDS: Altitude, Eurasian tree sparrow, Flight, Maximum performance, Wing loading

INTRODUCTION

Montane habitats pose diverse physiological challenges to animals relative to their basic physiological functions, including respiration, circulation and thermoregulation. Associated morphological adaptations in birds and mammals are known to include increases in the relative size of internal organs (e.g. Hammond et al., 2001) and in overall body size (e.g. Blackburn et al., 1999; Blackburn and Ruggiero, 2001). Moreover, animal flight at high altitude involves aerodynamic responses to hypodense conditions, along with enhanced oxygen delivery to flight muscles to compensate for its reduced atmospheric partial pressure (Altshuler and Dudley, 2006; Dillon et al., 2006). Increased metabolic demands in hypodense and

hypoxic air, in parallel with reduced capacity for aerodynamic force production, impose limits to locomotor performance at high altitudes (Altshuler and Dudley, 2003; Altshuler et al., 2004). Among different hummingbird species, high-altitude hovering is associated interspecifically with relatively longer wings, and also with a decline in maximum lifting capacity associated with limiting stroke amplitudes and thus with total force production by the wings (Altshuler and Dudley, 2003; Altshuler et al., 2004). Hummingbirds are nonetheless a highly derived avian lineage with wing kinematics and behavior radically different from all other birds, and the potential generality of the decline in elevation-dependent flight performance remains to be established for other small birds.

Intraspecific comparison of populations at different elevations reduces much of the genetic variability necessarily associated with among-species comparisons, particularly those of body size and its flight-related correlates. At present, intraspecific analysis of maximum flight capacity in birds is limited to studies on ruby-throated hummingbirds (*Archilochus colubris*) hovering in hypodense air. Hummingbirds with higher wing loadings failed in flight at relatively higher air densities under hyperoxic conditions, consistent with aerodynamic rather than metabolic limits on maximum hovering capacity (Chai and Dudley, 1996). We therefore hypothesized that for other avian taxa, the capacity for maximum vertical force production would similarly decline with elevation among different populations, but that the maximum aerodynamic wing loading attained during such flights, i.e. the ratio of effective body mass (including any supplemental load) to wing area, would be similar. To this end, we chose to study a representative passerine, the Eurasian tree sparrow [*Passer montanus* (Linnaeus 1758)], which is one of the most broadly distributed birds across the Eurasian continent (Summers-Smith, 2014).

The Eurasian tree sparrow has a wide elevational distribution from sea level to the Qinghai-Tibet Plateau (QTP), representing a vertical difference of more than 5000 m (Fu et al., 1998; Summers-Smith, 2014). The QTP is the largest high-altitude land area on the earth, and is characterized by hypobaric and cold habitats (Thompson et al., 2000), which likely impose selection on a variety of features of organismal design. Prior studies have found that Eurasian tree sparrows at different elevations express variable physiological and ecological traits (e.g. in stress response and life history characteristics; Li et al., 2011, 2013). As a human commensal, Eurasian tree sparrows settled on the QTP in parallel with human colonization approximately 20,000 years ago (Zhao et al., 2009; Y. H. Qu et al., unpublished data). Humans inhabiting the QTP also exhibit phenotypic and genetic adaptation to the low-oxygen and cold environments (see Beall, 2014). Comparable adaptation might be expressed in Eurasian tree sparrows on the QTP, which would shape flight-related adaptation to local elevation. As such, this short-lived species represents a convenient system in which to study intraspecific variation in flight performance (e.g. wingbeat kinematics and load-lifting capacity) and morphological

[1]Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, College of Life Sciences, Hebei Normal University, Shijiazhuang 050024, People's Republic of China. [2]Ocean College, Agricultural University of Hebei, Qinhuangdao 066003, People's Republic of China. [3]Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China. [4]Department of Integrative Biology, University of California, Berkeley, Berkeley, CA 94720, USA.

*Authors for correspondence (wings@berkeley.edu; lidongming@hebtu.edu.cn)

D.-M.L., 0000-0003-2759-3435

correlates (e.g. flight muscle mass, wing loading, and heart and lung indices) relative to both aerodynamic force production and metabolic capacity. In China, the Eurasian tree sparrow is a common resident species with large population sizes (Zhang and Zheng, 2010) and very small home ranges (~7600 m²; Pan and Zheng, 2003), so local adaptation in flight-related features is not likely to be overcome by migrants among populations at different elevations.

Here, we identify patterns of flight-related morphological variation, wingbeat kinematics and maximum load-lifting capacity among three populations of Eurasian tree sparrow covering ~3000 m in elevational range, which corresponds approximately to a 25% reduction in air density and oxygen partial pressure. We also include data on heart and lung mass to identify potential gross anatomical underpinnings to any increased capacity for oxygen transport and overall metabolic capacity.

MATERIALS AND METHODS
Animal collection and sampling sites
Thirty-three male and 29 female adult Eurasian tree sparrows were captured opportunistically in nature using mist nets during winter at three different sites in the People's Republic of China (see Table S1 and Fig. S1). Within 30 min post-capture, body mass was measured for each individual to the nearest 0.01 g using a portable digital balance. Birds were used in flight experiments within 2 h of capture.

Load-lifting assay
Birds were placed individually in a rectangular flight chamber (45×45×150 cm) made from transparent Plexiglas on two adjacent sides, and with a mesh covering on the top. Each tree sparrow was evaluated for asymptotic load-lifting capacity using an assay described elsewhere in detail (Chai and Millard, 1997; Chai et al., 1997; Altshuler and Dudley, 2003; Buchwald and Dudley, 2010)

Table 1. Morphological variables (means±s.d.) and statistical results among Eurasian tree sparrows across an altitudinal gradient including three sites (Shijiazhuang, 80 m; Zhangbei, 1400 m; Jiangxigou, 3230 m) in a linear mixed model with sex and site as fixed factors

Variable	80 m	1400 m	3230 m	Statistical results
Body mass (g)	19.17±1.82[a]	20.78±1.54[b]	23.31±1.30[c]	Site: $F_{2,55}$=27.088, $P<0.001$ Sex: $F_{1,55}$=3.409, $P=0.07$ Interaction: $F_{2,55}$=0.012, $P=0.988$
Wing length (cm)	9.38±0.24[a]	9.07±0.38[a]	9.63±0.36[b]	Site: $F_{2,55}$=13.554, $P<0.001$ Sex: $F_{1,55}$=5.334, $P=0.025$ Interaction: $F_{2,55}$=0.320, $P=0.728$
Wingtip shape (C2)	1.04±0.01[a]	1.08±0.01[a]	1.10±0.01[b]	Site: $F_{2,56}$=13.389, $P<0.001$ Sex: $F_{1,56}$=0.791, $P=0.378$ Interaction: $F_{2,56}$=1.249, $P=0.295$
Total wing area (cm²)	98.54±8.78[a]	97.64±8.28[a]	113.06±13.58[b]	Site: $F_{2,55}$=10.330, $P<0.001$ Sex: $F_{1,55}$=12.999, $P=0.001$ Interaction: $F_{2,55}$=2.915, $P=0.063$
Wing aspect ratio	1.79±0.14	1.90±0.14	1.94±0.18	Site: $F_{2,55}$=1.753, $P=0.183$ Sex: $F_{1,55}$=2.416, $P=0.126$ Interaction: $F_{2,55}$=0. 923, $P=0.403$
Wing loading (N m⁻²)	19.11±2.10[a]	20.77±1.75[b]	20.43±2.32[a,b]	Site: $F_{2,54}$=3.789, $P=0.029$ Sex: $F_{1,54}$=2.389, $P=0.128$ Interaction: $F_{2,54}$=2.390, $P=0.101$
Flight muscle mass (g)	1.68±0.24[a]	1.90±0.18[b]	2.14±0.21[c]	Site: $F_{2,56}$=19.144, $P<0.001$ Sex: $F_{1,56}$=5.699, $P=0.02$ Interaction: $F_{2,56}$=0.391, $P=0.678$
Relative flight muscle size (% body mass)	8.75±0.82	9.22±0.73	9.19±0.90	Site: $F_{2,55}$=2.029, $P=0.141$ Sex: $F_{1,55}$=1.846, $P=0.18$ Interaction: $F_{2,55}$=0.753, $P=0.476$
Mass of pectoralis major (g)	1.49±0.23[a]	1.72±0.17[b]	1.93±0.20[c]	Site: $F_{2, 56}$=20.416, $P<0.001$ Sex: $F_{1,56}$=6.316, $P=0.015$ Interaction: $F_{2,56}$=0.277, $P=0.759$
Mass of pectoralis minor (g)	0.19±0.02	0.18±0.03	0.21±0.02	Site: $F_{2,56}$=5.4, $P=0.007$ Sex: $F_{1,56}$=0.054, $P=0.817$ Interaction: $F_{2,56}$=1.025, $P=0.365$
Mass ratio of pectoralis major to pectoralis minor (%)	7.81±0.76[a]	9.93±2.49[b]	9.21±0.87[a,b]	Site: $F_{2,56}$=8.585, $P=0.001$ Sex: $F_{1,56}$=2.212, $P=0.143$ Interaction: $F_{2,56}$=0.486, $P=0.618$
Heart mass (g)	0.25±0.03[a]	0.32±0.04[b]	0.35±0.04[b]	Site: $F_{2,56}$=24.018, $P<0.001$ Sex: $F_{1,56}$=2.922, $P=0.093$ Interaction: $F_{2,56}$=1.003, $P=0.373$
Lung mass (g)	0.18±0.04[a]	0.21±0.03[a]	0.27±0.05[b]	Site: $F_{2,54}$=20.462, $P<0.001$ Sex: $F_{1,54}$=0.497, $P=0.484$ Interaction: $F_{2,54}$=0.814, $P=0.449$
Heart index (%)	1.33±0.1[a]	1.56±0.19[b]	1.48±0.18[a,b]	Site: $F_{2,55}$=7.562, $P=0.001$ Sex: $F_{1,55}$=0.341, $P=0.562$ Interaction: $F_{2,55}$=0.893, $P=0.415$
Lung index (%)	0.92±0.22[a]	1.00±0.14[a,b]	1.14±0.18[b]	Site: $F_{2,53}$=4.912, $P=0.011$ Sex: $F_{1,53}$=0.086, $P=0.770$ Interaction: $F_{2,53}$=0.757, $P=0.474$

Values with different letters in the same row are significantly different according to Bonferroni-adjusted *post hoc* comparisons (Table S2).

with minor modifications. Briefly, a thread with different plastic beads (each approximately 1 g in mass) was attached to the left tarsometatarsus of each bird. When released from the floor of the flight chamber, birds typically fly vertically towards the top of the chamber, asymptotically lifting more and more beads until a maximum load is attained, at which point they descend laterally towards the chamber walls. As these sparrows, like most small passerines, are capable of transient hovering, the load-lifting assay elicited behavior very similar to that of hummingbirds lifting increasing loads, with a smooth but asymptotically slowing descent to a transiently sustained peak (see Movies 1 and 2). The average air temperature during filming of load-lifting flight was 4.8°C (range: 3 to 6°C) in Shijiazhuang, −3.7°C (range: −5 to 1°C) in Zhangbei and −2.1°C (range: −4 to 2°C) in Jiangxigou.

Two synchronized high-speed video cameras (JVC, GC-P100BAC) were positioned on a tripod perpendicular to each transparent side of the flight chamber at a distance of 80 cm. One camera was operated at 50 frames s^{-1}, and was used to film the beads remaining on the flight chamber floor during load-lifting, and thus by subtraction to determine the total weight lifted by the bird (Fig. S2). The other camera, at a height from the chamber floor of 80 cm, was operated at 250 frames s^{-1} and was used to obtain wingbeat kinematics (i.e. wingbeat frequency and stroke amplitude). In take-off and at maximum load-lifting, birds beat their wings using a powered and vertically oriented downstroke, with the body axis

also being vertically aligned. Stroke amplitude was derived from video images in which the wings were located at the extreme positions of the wingbeat (between downstroke and upstroke positions; see Fig. S3 and Movie 2); a mean value was calculated from three to five separate measurements within each bout of maximal loading within the final 0.5 s of peak lifting performance. Wingbeat frequency was determined by the interaction frequency between wing motions and the camera filming speed for the same measurement period. A time-averaged wingbeat frequency was calculated. Multiple ascending flights were recorded for each bird (5.8 flights on average), and the maximum weight lifted within the series was assumed to indicate the limit to lifting performance.

Immediately following load-lifting trials, birds were euthanized with phenobarbitone (7.5 μl g^{-1} body mass). The pectoralis major, pectoralis minor, and the whole heart and lungs (following blotting to remove blood) were immediately excised and weighed using a digital balance sensitive to 0.1 mg. The right wing of each bird was photographed for measurements of the wing area S and wing length R (ImageJ, National Institutes of Health, Bethesda, MD, USA); total wing area S is given by twice the area of the right wing. The wing aspect ratio is given by $4R^2/S$, and wing loading was calculated by dividing the body weight by the wing area (Andrews et al., 2009). Wingtip shape (i.e. C2, a measure of roundness) was calculated using a standard metric (Lockwood et al., 1998) from the lengths of eight primary feathers (each measured to 0.1 mm). Maximum wing
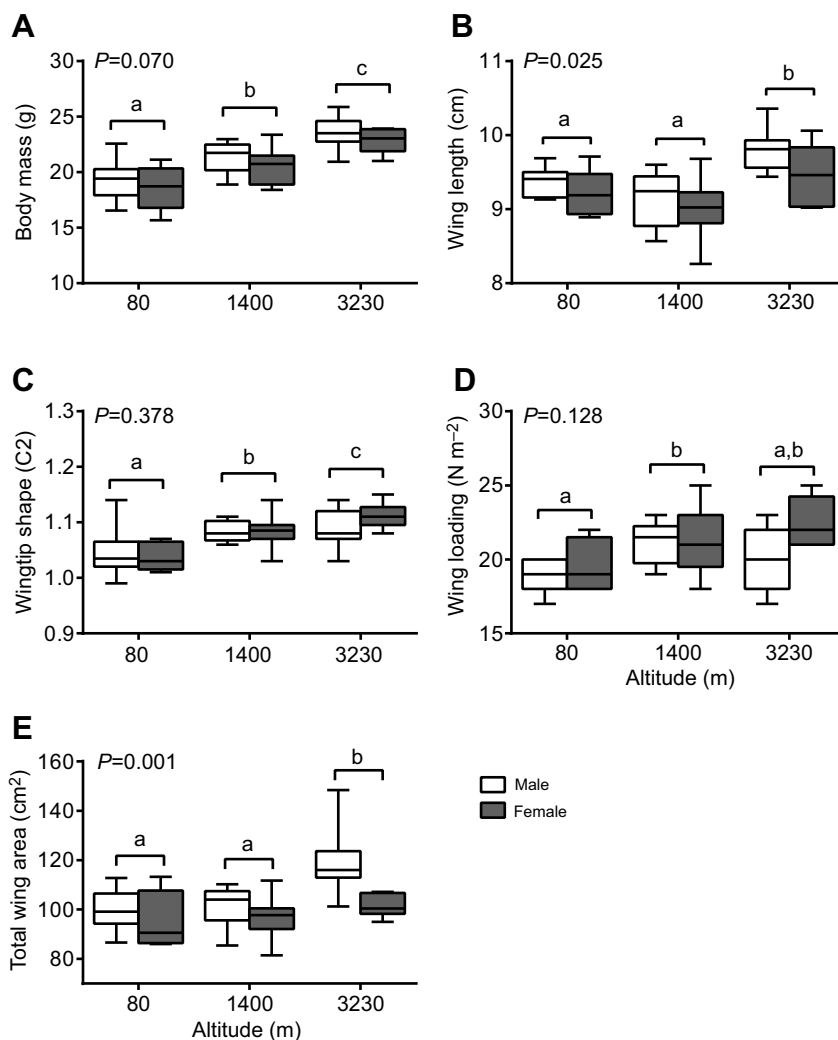

Fig. 1. Comparisons of morphological parameters for Eurasian tree sparrows by altitude (Shijiazhuang, 80 m; Zhangbei, 1400 m; Jiangxigou, 3230 m) and sex. (A) Body mass (g), (B) wing length (mm), (C) wingtip shape (C2), (D) wing loading (N m^{-2}) and (E) total wing area (cm^2). The P-values of comparisons between sexes are shown. Values depicted are the median for each group with the upper and lower limits of the box representing the 75th and 25th percentiles, respectively. The error bars represent the 95th and the 5th percentiles. Letters above bars represent significant differences among groups (P<0.05) based on Bonferroni-adjusted *post hoc* tests. See Table S2 for further details.
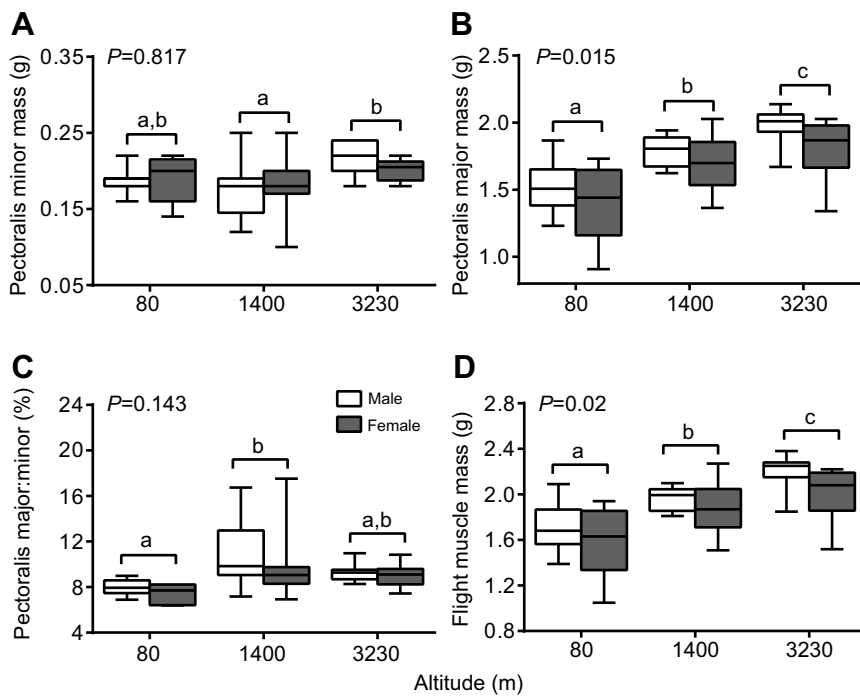
Fig. 2. Comparisons of flight muscle for Eurasian tree sparrows by altitude (Shijiazhuang, 80 m; Zhangbei, 1400 m; Jiangxigou, 3230 m) and sex. (A) Pectoralis minor mass (g), (B) pectoralis major mass (g), (C) mass ratio of pectoralis major and minor (%) and (D) flight muscle mass (g). The *P*-values of comparisons between sexes are shown. Values depicted are the median for each group with the upper and lower limits of the box representing the 75th and 25th percentiles, respectively. The error bars represent the 95th and the 5th percentiles. Letters above bars represent significant differences among groups (*P*<0.05) based on Bonferroni-adjusted *post hoc* tests. See Table S2 for further details.

loading was calculated by dividing the sum of body weight and loading weight by the total wing area. Relative flight muscle size was calculated by dividing flight muscle mass (i.e. the sum of the pectoralis major and minor) by the body mass (Wright et al., 2014). Heart and lung indices were calculated by dividing the heart mass and lung mass by the body mass, respectively (Vinogradov and Anatskaya, 2006; Wright et al., 2014).

All protocols were approved by the Ethics and Animal Welfare Committee (no. 2013-6) and by the Institutional Animal Care and Use Committee (HEBTU2013-7) of Hebei Normal University, China, and were carried out under the auspices of scientific collecting

permits issued by the Departments of Wildlife Conservation (Forestry Bureau) of Hebei and Qinghai Provinces, China.

Statistical analysis

To examine potential differences in all morphological and functional variables, we used a linear mixed model (LMM) fitted with the restricted maximum-likelihood (REML) method to test the fixed effects of study site, sex and the interaction between site and sex. We used SPSS 21.0 software to fit LMMs and to estimate *F* statistics, denominator degrees of freedom and *P*-values. Differences between pairs of means were identified by Bonferroni-adjusted *post hoc* tests
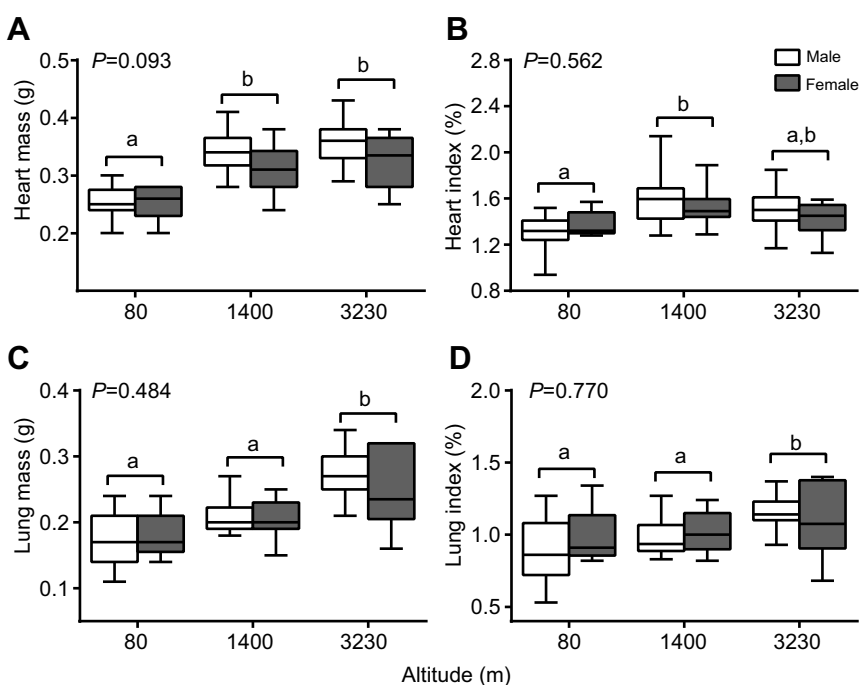

Fig. 3. Comparisons of heart and lung for Eurasian tree sparrows by altitude (Shijiazhuang, 80 m; Zhangbei, 1400 m; Jiangxigou, 3230 m) and sex. (A) Heart mass (g), (B) heart index (%), (C) lung mass (g) and (D) lung index (%). The *P*-values of comparison between sexes are shown. Values depicted are the median for each group with the upper and lower limits of the box representing the 75th and 25th percentile, respectively. The error bars represent the 95th and the 5th percentiles. Letters above bars represent significant differences among groups (*P*<0.05) based on Bonferroni-adjusted *post hoc* tests. See Table S2 for further details.

Table 2. Functional variables (means±s.d.) and statistical results among Eurasian tree sparrows across an altitudinal gradient including three sites (Shijiazhuang, 80 m; Zhangbei, 1400 m; Jiangxigou, 3230 m) in a linear mixed model with sex and site as fixed factors

Variable	80 m	1400 m	3230 m	Statistical results
Maximum load (g)	26.28±2.61	24.76±3.91	25.13±2.90	Site: $F_{2,52}$=0.252, P=0.778 Sex: $F_{1,52}$=0.109, P=0.742 Interaction: $F_{2,52}$=0.511, P=0.603
Stroke amplitude (deg)	158.35±3.91	159.76±4.50	157.70±2.65	Site: $F_{2,52}$=1.876, P=0.163 Sex: $F_{1,52}$=0.119, P=0.731 Interaction: $F_{2,52}$=0.949, P=0.394
Wingbeat frequency (Hz)	23.58±1.12[a]	25.52±0.92[b]	24.30±0.86[b]	Site: $F_{2,52}$=3.333, P=0.043 Sex: $F_{1,52}$=7.441, P=0.009 Interaction: $F_{2,52}$=0.805, P=0.452
Loading weight/wing area (N m^{-2})	23.69±2.64	25.39±5.05	22.07±3.46	Site: $F_{2,51}$=1.624, P=0.207 Sex: $F_{1,51}$=3.215, P=0.079 Interaction: $F_{2,51}$=0.287, P=0.752
Maximum wing loading (N m^{-2})	42.70±4.27	45.34±4.69	42.50±5.50	Site: $F_{2,50}$=2.074, P=0.136 Sex: $F_{1,50}$=0.095, P=0.759 Interaction: $F_{2,50}$=0.092, P=0.912
Maximum load/body mass	1.26±0.13[a]	1.19±0.22[a,b]	1.078±0.11[b]	Site: $F_{2,51}$=3.745, P=0.030 Sex: $F_{1,51}$=0.514, P=0.477 Interaction: $F_{2,51}$=0.727, P=0.489

Values with different letters in the same row are significantly different according to Bonferroni-adjusted *post hoc* comparisons (Table S2).

based on model-predicted estimated marginal means in LMMs. Differences were considered significant at $P<0.05$.

RESULTS

Variation in morphological parameters with elevation

All morphological variables, with the exception of relative flight muscle mass and wing aspect ratio, varied significantly across elevation (Table 1, Figs 1–3). Sparrows were heavier and had more rounded wings at higher elevations (Fig. 1A,C, Table S2). Sparrows at 3230 m of altitude had longer wings and greater wing areas relative to their low-altitude counterparts (Fig. 1B,E, Table S2). Sparrows at 1400 m of altitude exhibited greater values of wing loading than those from 80 m of altitude (Fig. 1D, Table S2).

Variation in flight muscle with elevation

Total flight muscle mass increased with elevation, as did the mass of the pectoralis major (Fig. 2B,D, Table S2). Sparrows at 3230 m of altitude exhibited a relatively larger pectoralis minor, whereas those at 1400 m had greater mass ratio for the pectoralis major relative to pectoralis minor when compared with those from other altitudes (Fig. 2A,C, Table S2). Relative flight muscle mass, however, showed no change with altitude (Table 1), indicating isometric scaling as total body mass changed. Both heart and lung masses tended to increase with elevation, as did their relative size (Fig. 3, Table S2). Relative lung size in the sparrows was particularly high at the 3230 m site (Fig. 3D, Table S2).

Males and females differed significantly only in wing length, wing area, the mass of the pectoralis major and total flight muscle

(Table 1). Male sparrows had longer wings, greater wing areas, total flight muscle mass and pectoralis major mass than females (Figs 1B, E and 2B,D). There were no significant interaction effects between study site and sex (Table 1).

Functional variation with elevation

Sparrows at higher elevations lifted relatively less weight relative to their body weight, independently of sex (Table 2, Fig. 4A). No significant differences were found in maximum load, the ratio of maximum load to wing area, stroke amplitude or maximum wing loading across elevations (Table 2). By contrast, wingbeat frequency showed a slight but significant increase with elevation, along with significant differences between the sexes (Table 2, Fig. 4B). There were no interaction effects between site and sex for any of the measured functional variables (Table 2).

DISCUSSION

Here, we have identified patterns of intraspecific variation in a suite of flight-related morphological and physiological variables. Eurasian tree sparrows at higher elevations tend to be heavier with longer wings and greater wing loadings and wing aspect ratios, trends that correspond generally to those documented previously in the avian flight literature (see Dudley and Chai, 1996; Altshuler and Dudley, 2006). Sparrows at higher altitudes also exhibit more rounded wings, which may contribute to enhanced maneuverability (see Lockwood et al., 1998; Arizaga et al., 2006). Absolute flight muscle mass also increases at higher altitudes, principally because of increases in the pectoralis major (i.e. the primary downstroke muscle in passerines;
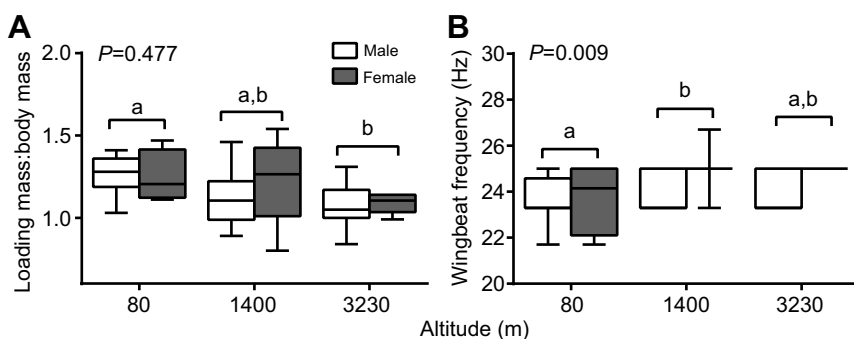

Fig. 4. Comparisons of flight-related parameters Eurasian tree sparrows by altitude (Shijiazhuang, 80 m; Zhangbei, 1400 m; Jiangxigou, 3230 m) and sex. (A) Ratio of loading mass to body mass and (B) wingbeat frequency (Hz). The P-values of comparisons between sexes are shown. Values depicted are the median for each group with the upper and lower limits of the box representing the 75th and 25th percentiles, respectively. The error bars represent the 95th and the 5th percentiles. Letters above bars represent significant differences among groups (P<0.05) based on Bonferroni-adjusted *post hoc* tests for loading mass:body mass, and least-significant difference *post hoc* tests for wingbeat frequency. See Table S2 for further details.

Norberg, 1990), although relative flight muscle mass shows no change. Relative heart and lung masses, by contrast, tend to increase significantly at higher field sites (Fig. 3B,D), suggesting a concomitant physiological response to environmental hypoxia (Dunson, 1965; Carey and Morton, 1976; Monge and León-Velarde, 1991; Scott, 2011). In aggregate, these morphological trends represent a sustained pattern of phenotypic response to long-term montane residence by this species.

Some of these trends may reflect adaptation to the lower air temperatures characteristic of higher elevations. For example, relative heart mass (but not relative lung mass) increases in the same species at a high-latitude location relative to a comparison population at a lower latitude, as do resting metabolic rates and various enzyme activities in the liver and muscle, although body mass stays approximately constant (Zheng et al., 2014). The three sites under consideration here lie within 5° of latitude from each other, but likely exhibit substantial differences in mean daily temperature (e.g. >10°C; see Figs S1 and S4). For hummingbirds, interspecific comparisons indicate that heavier species tend to be found at higher elevations (Altshuler et al., 2004, 2010). A similar pattern of body mass increase characterizes Andean passerines and ducks across elevation (Blackburn and Ruggiero, 2001; Gutiérrez-Pinto et al., 2014). Such increases in body mass may simply reflect associated thermal advantages of large body size in lower air temperatures (e.g. Teplitsky and Millien, 2014), and may not reflect selection on flight performance. Our load-lifting measurements were all made in the winter at fairly low air temperatures within 8°C of each other (see Materials and methods); the lowest temperatures were predictably at the highest elevation site, but the endothermic capacity of all birds likely renders high levels of performance relatively independent of ambient temperature (e.g. Chai, 1998).

By contrast, maximum lifting performance by Eurasian tree sparrows declines sharply at higher elevation. The maximum lifted load declines relative to body mass (Fig. 4A), in parallel with a slight but significant increase in wingbeat frequency but with an invariant maximum stroke amplitude (Table 2). Interspecifically, hummingbirds exhibit a similar decline with body size in their relative maximum load-lifting capacity performance (Altshuler et al., 2004). In neither Eurasian tree sparrows nor hummingbirds does relative flight muscle mass increase with elevation, which could otherwise potentially compensate for the increased power demands of hypodense air. Molting Eurasian tree sparrows are known to increase their relative pectoral muscle size, enabling escape flights dynamically comparable to those in non-molting conditions (Lind, 2001; Lind and Jakobsson, 2001). Such an increase is not, however, characteristic among sparrow populations at different elevations (Table 1), consistent with a relative decline in load-lifting performance. At higher elevations, both male and female sparrows increased wingbeat frequency during load-lifting (as do hummingbirds in an interspecific comparison; Altshuler and Dudley, 2003); but the magnitude of such increases was only on the order of 1 Hz (Table 2), and as such is small relative to the parallel decline in air density over 3000 m.

The Eurasian tree sparrow is generally considered to be sexually monomorphic (Summers-Smith, 2014), and we similarly found no significant differences between males and females in many of the examined morphological traits examined (Table 1). However, male sparrows do tend to have longer wings (Fig. 1B; see also Mónus et al., 2011), and we also found that they have greater wing area and a greater pectoralis major mass and total flight muscle mass relative to females (Figs 1E and 2B,D). Functional performance in maximum load-lifting between the sexes was, however, equivalent, with no

differences in either maximum load or in the maximum effective wing loading thereby attained (Table 2). Female sparrows effected such lifting performance with consistently shorter and smaller wings and with higher wingbeat frequencies relative to males (Fig. 4B), albeit with equivalent stroke amplitudes. This capacity for an increase in flapping frequency may, in part, be associated with their smaller wings relative to male sparrows. Nonetheless, given similarities in body mass, relative flight muscle mass, and in the unloaded values for wing loading, convergence between the sexes in maximum flight performance across an altitudinal gradient is not surprising.

Conclusions

We have identified a suite of flight-related morphological and functional variables that change systematically across elevations for the Eurasian tree sparrow, a non-migratory passerine. High-elevation sparrows exhibit greater body mass and longer and more rounded wings, but a relatively reduced capacity for maximum load-lifting. Populations of these sparrows are likely to be genetically distinct over the large altitudinal range considered here, and we correspondingly suggest that genomic approaches now be used to identify candidate genes involved in regulation of body size, and potentially in flight muscle isoforms, which could influence maximum take-off performance. Because maximum lifting capacity (as assayed by asymptotic loading; Buchwald and Dudley, 2010) likely reflects an important component of translational agility and thus escape performance in vertical takeoff, the sparrow populations here should also exhibit substantial differences in their unloaded take-off mechanics, along with variation in behavioral propensity to volitionally engage in escape flight. These possibilities are amenable to field investigation.

Competing interests
The authors declare no competing or financial interests.

Author contributions
D.-M.L. and R.D. conceived of, designed and coordinated the study, and also directed the writing of the manuscript; Y.-F.S. and Z.-P.R. collected field data and implemented data analyses. Y.-F.W. and F.-M.L. advised and also helped to revise the manuscript prior to submission.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.142216.supplemental

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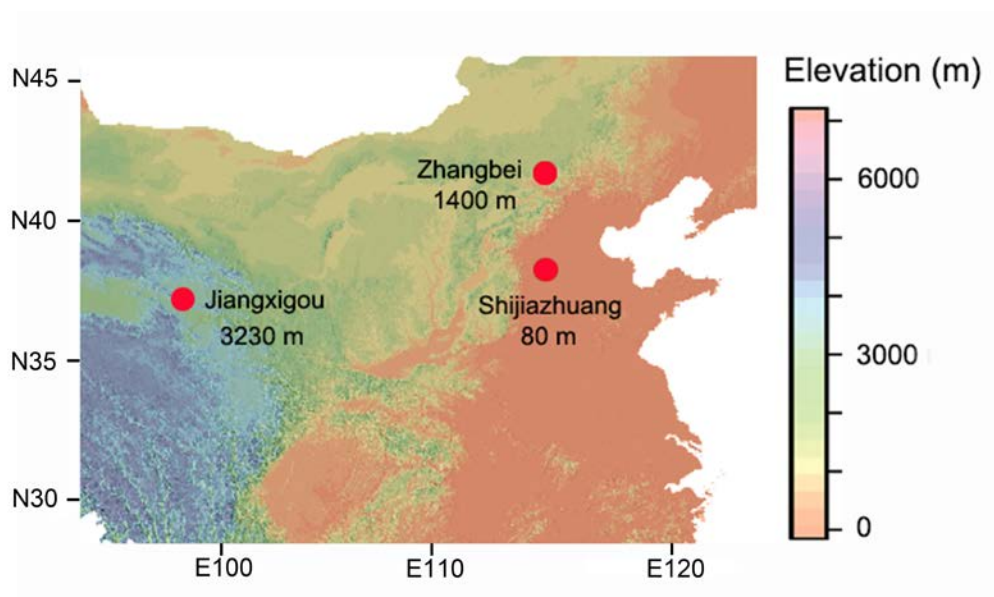
SUPPLEMENTARY INFORMATION

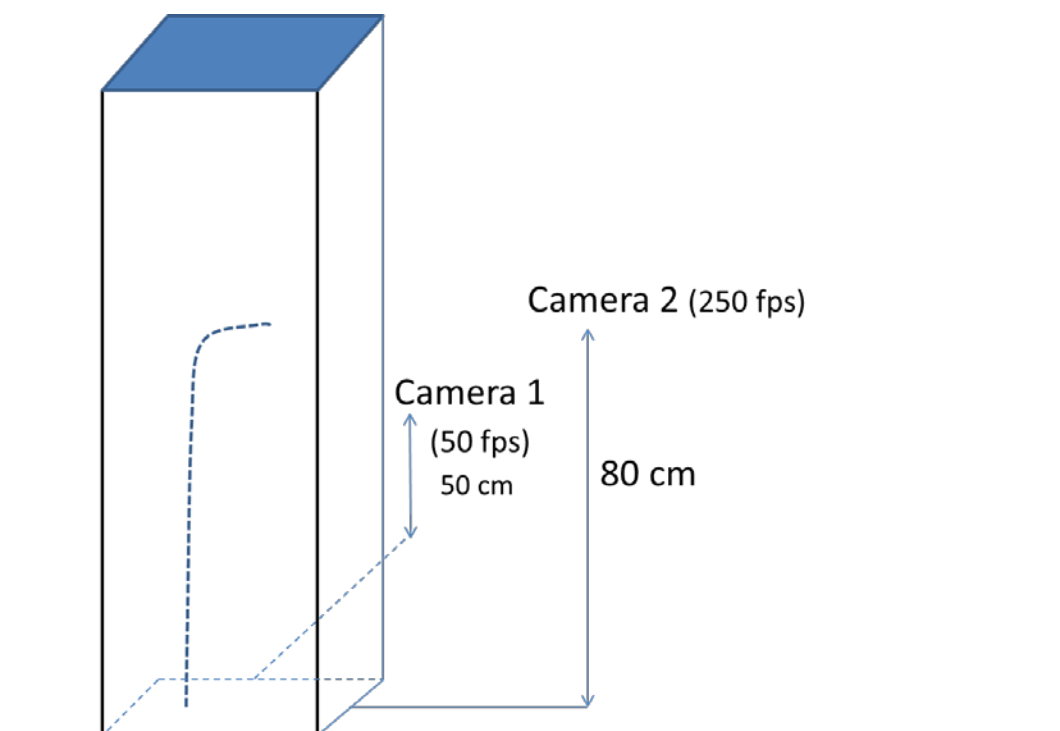**Fig. S1. Sampling sites (red dots) and altitudinal information (Shijiazhuang, Zhangbei and Jiangxigou).**

Fig. S2. Schematic diagram of the flight chamber with the two recording cameras
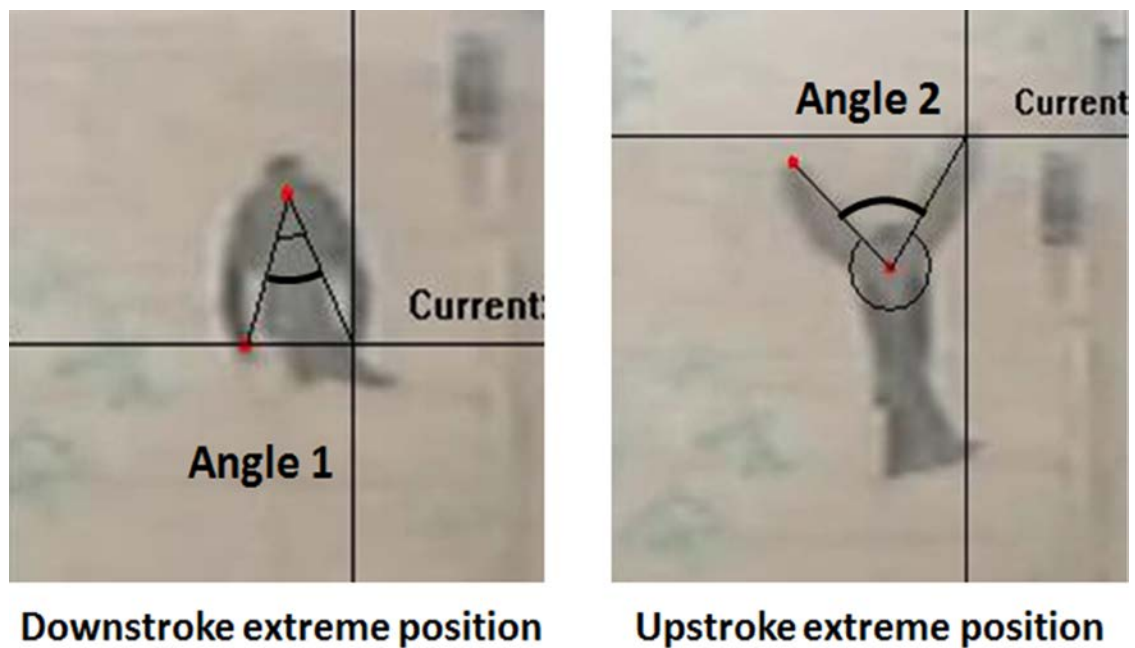
Fig. S3. Angle estimation method for stroke amplitude. We calculated the angle between the two wings at the extreme positions of downstroke (Angle 1) and upstroke (Angle 2). The angle of stroke amplitude is estimated by [360˚-(angle 1 + angle 2)]/2.
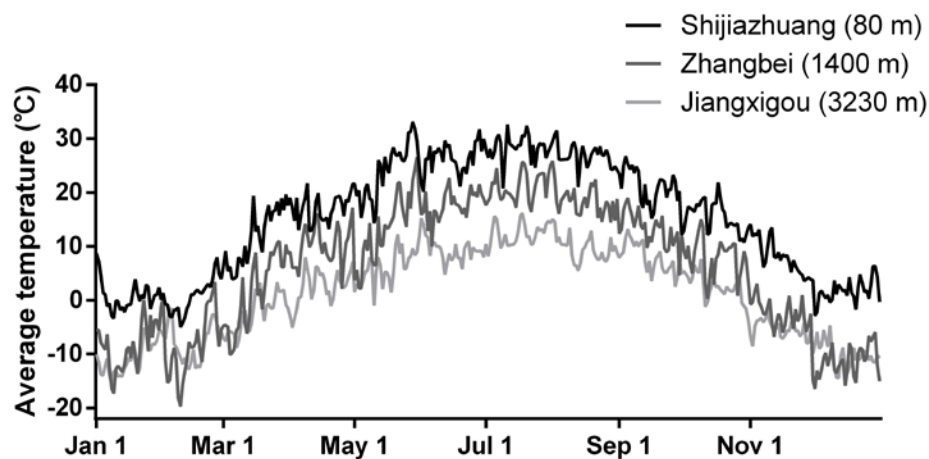

Fig. S4. Mean daily temperature of 2014 at the three sampling sites (Shijiazhuang, Zhangbei and Jiangxigou).

SUPPLEMENTARY INFORMATION

Table S1. Geographical and sampling information for three populations of Eurasian tree sparrows.

Sampling site	GPS location	Altitude (m)	Sampling dates	Average air pressure	Sample size
Shijiazhuang (Hebei Province)	N37°59.898′, E114°31.090′	80 m	January 16 –18, 2015	100 kPa	17 (male, 5; female, 12)
Zhangbei (Hebei Province)	N41°10.169′, E114°46.711′	1400 m	January 6 –10, 2015	86 kPa	28 (male, 10; female, 18)
Jiangxigou (Qinghai Province)	N36°37.098′, E100°16.690′	3230 m	January 24 to February 14, 2015	70 kPa	17 (male, 11; female, 6)

Table S2. Bonferroni adjusted *P*-values for post-hoc comparisons of morphological and functional variables between sites in Eurasian tree sparrows using a linear mixed model based on model-predicted estimated marginal means.

Variable	Altitude	80 m	1400 m
Body mass	1400 m	**0.002**	-
	3230 m	**<0.001**	**<0.001**
Wing length	1400 m	0.199	-
	3230 m	**0.020**	**<0.001**
Wingtip shape (C2)	1400 m	**0.001**	-
	3230 m	**<0.001**	0.295
Wing area	1400 m	1.0	-
	3230 m	**0.001**	**<0.001**
Wing loading	1400 m	**0.042**	-
	3230 m	0.069	1.0
Flight muscle mass	1400 m	**0.001**	-
	3230 m	**<0.001**	**0.013**
Mass of pectoralis major	1400 m	**<0.001**	-
	3230 m	**<0.001**	**0.027**
Mass of pectoralis minor	1400 m	0.663	-
	3230 m	0.253	**0.005**
Mass ratio of p. major to p. minor	1400 m	**<0.001**	-
	3230 m	0.082	**0.298**
Heart mass	1400 m	**<0.001**	-
	3230 m	**<0.001**	0.620
Lung mass	1400 m	0.069	
	3230 m	**<0.001**	**<0.001**
Heart index	1400 m	**0.001**	
	3230 m	0.165	0.254
Lung index	1400 m	0.966	
	3230 m	**0.012**	0.062
Wingbeat frequency *	1400 m	**0.029**	
	3230 m	**0.022**	0.765
Maximum load /body mass	1400 m	0.578	-
	3230 m	**0.027**	0.289

* Unadjusted p-value from Least-significant Difference (LSD) method

Movie 1. Loading lift video of Eurasian tree sparrow (Camera 1 for framing loading beads set at 50 fps).

Movie 2. Loading lift video of Eurasian tree sparrow (Camera 2 for amplitude and wingbeat frequency set at 250 fps).