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THE MECHANISTIC BASIS OF AEROBIC PERFORMANCE VARIATION IN RED JUNGLEFOWL

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Summary

We examined aerobic performance, organ and muscle mass and enzymatic activity in red junglefowl (*Gallus gallus*). We tested three models of performance limitation (central limits, peripheral limits, symmorphosis) and explored relationships between basal metabolic rate (BMR), aerobic capacity ($\dot{V}_{O_2max}$) and social rank. Males had a lower BMR, a higher $\dot{V}_{O_2max}$ and a greater aerobic scope than females. Females possessed larger peritoneal and reproductive organs, while males had larger hearts, lungs and leg muscles. In females, BMR was correlated with spleen mass and $\dot{V}_{O_2max}$ was correlated with hematocrit and large intestine mass. Male BMR was correlated with intestinal tract and lung mass, and $\dot{V}_{O_2max}$ was correlated with heart and pectoralis mass. Male citrate synthase activity averaged 57 % higher than that of females and was correlated with $\dot{V}_{O_2max}$ (this correlation was not significant in females). Female social status was not correlated with any variable, but male dominance was associated with higher aerobic scope, larger heart and lungs, smaller peritoneal organs and greater leg citrate synthase activity. We conclude that aerobic capacity is controlled by system-wide limitations (symmorphosis) in males, while in females it is controlled by central organs. In neither sex is elevated aerobic capacity associated with increased maintenance costs.

Key words: aerobic performance, citrate synthase, symmorphosis, metabolic rate, social rank, muscle mass, red junglefowl, *Gallus gallus*.

Introduction

Natural selection operates on organismal-level traits that are usually manifestations of the integrated functioning of a suite of organs and organ systems. Some of the most intensively studied of these traits are indices of overall physiological vigor or ‘quality’, such as locomotor performance (sprint speed, endurance, etc.) and aerobic capacity (Taylor and Weibel, 1981; Weibel, 1984; Chappell and Snyder, 1984; Garland and Else, 1987; Hammond et al., 1994; Chappell and Bachman, 1995).

Theoretically, locomotor and aerobic performance limits might be set by peripheral effectors (primarily skeletal muscle) or by the visceral infrastructure (the digestive, pulmonary, cardiovascular or excretory organs) that supports the peripheral effectors. These views represent the ‘peripheral limitation hypothesis’ and the ‘central limitation hypothesis’, respectively (Peterson et al., 1990; Weiner, 1993). An alternative model is that all components of whole-animal performance traits are optimally scaled such that no one component is limiting and there is no expensive ‘excess capacity’. This concept, called ‘symmorphosis’, has been extensively promoted for the mammalian oxygen delivery system (e.g. Taylor and Weibel, 1981; Weibel, 1984; Weibel et al., 1991). Nevertheless, there is surprisingly little consensus on what organ systems or other factors limit aerobic or locomotor performance, even for the much-studied aerobic pathway in mammals.

These questions can be investigated by making use of the natural variation typical of essentially all physiological traits. The repeatability and heritability of inter-individual differences are critical in determining how (or if) a performance trait can be affected by natural selection (e.g. Jayne and Bennett, 1990; Garland and Bennett, 1990). From a mechanistic perspective, analyses of variation at different levels of integration (e.g. enzymes, organelles, cells, organs, organ systems and the intact animal) can provide useful insights into the functional basis of whole-animal performance (e.g. Else and Hulbert, 1981; Hulbert and Else, 1981; Chappell and Snyder, 1984; Garland, 1984; Weibel, 1984; Bennett, 1997).

We have used this approach in a study of aerobic performance in red junglefowl (*Gallus gallus*). These birds show substantial and repeatable variation in aerobic capacity (Chappell et al., 1996, 1998, 1999b). In addition to variability within each sex, the species exhibits striking sexual dimorphism in aerobic performance, with the aerobic capacity of males greatly exceeding that of females (Chappell et al., 1996). An intuitively attractive explanation for this difference is that neither sex engages in sustained flight but that, unlike

females, males must engage in sustained and vigorous fights and displays to compete for matings and social status (Chappell et al., 1996, 1997b).

Using both inter- and intrasexual performance differences, we tested the central limitation, peripheral limitation and symmorphosis models. The peripheral limitation model predicts strong correlations between effector organ properties (the mass and aerobic enzyme capacities of skeletal muscle) and aerobic performance, but not between visceral organ properties and performance. Conversely, the central limitation hypothesis predicts positive correlations between properties of the visceral organs (mass of digestive organs, heart, lungs, reproductive organs, etc.) and aerobic performance, while the symmorphosis concept predicts correlations between performance and both skeletal muscle and visceral organ properties. We also tested the concept, implicit to both the central limitation and symmorphosis hypotheses, that basal metabolic rate (BMR) and aerobic capacity should be positively correlated since a high aerobic performance requires a larger 'investment' in metabolically expensive visceral organs, which elevates BMR (e.g. Kersten and Piersma, 1987; Peterson et al., 1990; Daan et al., 1990a; Weiner, 1993, Konarzewski and Diamond, 1995; Ricklefs et al., 1996; see also Bennett and Ruben, 1979).

Finally, we used our results to test whether social interactions are correlated with aerobic performance and investment in central and peripheral organs. If high aerobic performance in males is an evolutionary consequence of intense intermale combat, then one would expect both correlations between variation in the aerobic capacity of males and their ability aggressively to dominate other males and correlations between the mechanistic basis of aerobic performance (organ properties) and aggressive success.

Materials and methods

Animals and treatment groups

We studied red junglefowl (*Gallus gallus* L.) from a captive colony at the University of California, Riverside, USA. The ancestral stock was obtained in the late 1980s from a feral flock at the San Diego Zoo in southern California; subsequently, they have been reared using a minimum of 50 breeding males and 50 breeding females each generation (the zoo flock was descended from wild-caught birds imported from southern Asia in the 1940s). The population has not experienced artificial selection for activity patterns, physiology or reproductive biology.

For this study, chicks were hatched in incubators and kept indoors in brooders for 6 weeks, after which they were maintained under semi-natural conditions (outdoor pens exposed to ambient weather and photoperiod; Zuk et al., 1990, 1995). At approximately 4 months of age, the birds were separated into male–female pairs. At approximately 9 months, the birds were recombined into groups of three females and two males (which we term 'test flocks') for the social rank study. All birds in this study were hatched in the same year.

Social rank

We formed 18 test flocks and housed them in cages (2.5 m×1.1 m×1.25 m) constructed from wood and chicken wire. All members of test flocks had been visually isolated from each other for 1 month prior to flock formation. Each bird was marked with a unique combination of a colored leg band and a metal identification band.

Dominance status within male dyads and female triads was scored from similar criteria. We observed test flocks from a distance of 4–6 m and recorded three behavior patterns: fighting, pecking and displacement (Zuk et al., 1998). During a fight, both birds attacked, and the one retreating was scored as subordinate in that interaction. Pecks were usually aimed at or near the opponent's head; again, the bird being pecked was considered the subordinate if it retreated. In displacement, one bird threatened another, as demonstrated by chasing, sometimes accompanied by raised hackle feathers. The object of the chase retreated and was deemed subordinate. On the basis of at least 10 interactions per pair, the females were ranked from 1 to 3, 1 being the dominant and 3 the most subordinate. Males were ranked as either dominant or subordinate (hierarchies were unambiguous in all 18 test flocks).

Aerobic capacity, BMR and aerobic scope

We measured the aerobic capacities of the birds by means of forced exercise on an enclosed, motorized running wheel (76 cm in diameter and 42 cm wide) that also functioned as an open-circuit respirometer (Chappell et al., 1996, 1997a, 1998). This method reliably elicits maximal $\dot{V}_{O_2}$ (Chappell et al., 1996). Incurrent flow rates of dry air were 40–44 l min^{-1} STP, metered to ±2 % with Tylan mass flow controllers. Excurrent air was dried, scrubbed of CO_2 and analyzed for oxygen content (±0.0015 %; Applied Electrochemistry S-3A). Oxygen concentrations were recorded on a Macintosh computer. We ran birds at gradually increasing speeds until they could no longer maintain position and $\dot{V}_{O_2}$ did not increase with increasing speed. Birds invariably showed signs of exhaustion by this time, but none was injured. All birds both flapped and ran vigorously at the start of wheel motion. Complete measurements lasted 6–12 min (2.5–8 min of actual exercise), and we did not use data from tests in which birds could not run for at least 2 min. A blind trial arrangement ensured that the operator of the running wheel did not know the social rank of birds being tested.

We used the 'instantaneous' calculation (Bartholomew et al., 1981) to compensate for the mixing characteristics of the large-volume wheel and accurately resolve short-term changes. We computed maximal aerobic rate ($\dot{V}_{O_2max}$) as the highest instantaneous $\dot{V}_{O_2}$ averaged over a continuous 1 min interval.

We measured each bird's basal metabolic rate (BMR; ml O_2 min^{-1}) during the night following $\dot{V}_{O_2max}$ testing. Birds had been fasted for approximately 8 h at the beginning of BMR measurements (approximately 18:00 h local time), which continued for 12 h until the next morning. We used the same equipment for BMR data as was used for $\dot{V}_{O_2max}$ studies, except that Plexiglas boxes (volume 20–50 l) were used as

respirometers, flow rates were 6–12 l min^{-1} STP, and we did not use the instantaneous correction. Up to three birds were tested simultaneously in separate chambers. A computer-controlled bank of solenoid valves produced a 3 min reference reading every 2 h throughout the night. We computed BMR as the lowest $\dot{V}_{O_2}$ averaged over a continuous 10 min interval (Chappell et al., 1996).

As indices of the expansibility of aerobic metabolism, we calculated both the factorial scope ($\dot{V}_{O_2max}$/BMR) and the absolute scope ($\dot{V}_{O_2max}$ per gram body mass minus BMR per gram body mass). The factorial scope is the magnitude of maximal aerobic output scaled to maintenance costs, and the absolute scope reflects the energy available for uses other than maintenance (exercise, thermoregulation, reproduction, etc.; but see Ricklefs et al., 1996).

Hematocrit and enzyme studies

On the morning following BMR tests, blood samples (100–150 µl) were collected from the alar vein into heparinized microhematocrit tubes, which were sealed and centrifuged. Hematocrit was determined to the nearest 1 %.

Immediately after blood sampling, birds were killed instantaneously by cervical dislocation and weighed to ±1 g. We immediately took muscle samples (0.5–1.2 g) from the left pectoralis and the left leg (care was taken to sample from the same location on each bird). The samples were weighed to ±0.1 mg, sealed in cryogenic vials, flash-frozen in liquid nitrogen within 4 min after death and stored at –80 °C.

We measured the activities of a glycolytic enzyme (hexokinase) and an aerobic enzyme (citrate synthase) in the muscle samples. Reagents were purchased from Fisher Scientific (Pittsburgh, PA, USA) except for the following: ATP, EDTA, L-malate, NAD, NADP, bovine serum albumin (BSA), Triton X-100 and glucose-6-phosphate dehydrogenase, were purchased from Sigma Chemical Company (St Louis, MO, USA); and malate dehydrogenase was purchased from CalBiochem (La Jolla, CA, USA). Muscle extraction buffer contained 100 mmol l^{-1} sodium, potassium phosphate buffer and 2 mmol l^{-1} EDTA adjusted to pH 7.2. The hexokinase reaction mixture contained 5 mmol l^{-1} $MgCl_2$, 100 mmol l^{-1} Tris-HCl, 1 mmol l^{-1} glucose, 5 mmol l^{-1} ATP, 100 µmol l^{-1} NADP, 0.05 % BSA, 0.5 % Triton X-100 and 0.3 units ml^{-1} glucose-6-phosphate dehydrogenase adjusted to pH 8.0. The citrate synthase reaction mixture contained 100 mmol l^{-1} Tris-HCl, 2.5 mmol l^{-1} EDTA, 5 mmol l^{-1} NAD, 60 mmol l^{-1} L-malate, 0.02 mg ml^{-1} BSA and 60 units ml^{-1} malate dehydrogenase adjusted to pH 8.05.

Muscle samples were immediately frozen until analysis. A small piece of tissue was excised, weighed and homogenized in a glass/glass Duall homogenizer with 78 volumes of ice-cold muscle extraction buffer. The homogenized suspension was then stirred for 15 min on ice, and the concentration was adjusted with muscle extraction buffer to a final value of 12.82 µg ml^{-1}. Homogenates were kept on ice until used in the enzyme assays.

Hexokinase activity was quantified in a Hitachi F-2000 fluorimeter by measuring the reduction of NADP as in a procedure from Passoneau and Lowry (1993) except that the temperature was 30 °C rather than 25 °C. The reaction mixture (1 ml) was added to a quartz fluorimeter cuvette, and the reaction was started by adding 25 µl of a 12.82 µg ml^{-1} muscle sample in extraction buffer. The production of NADPH was monitored at an excitation wavelength of 343 nm and an emission wavelength of 455 nm. The fluorescence increase was measured for 5 min, and the slope was used to determine hexokinase activity (expressed as µmoles of glucose converted to 6-P-gluconolactone per gram of muscle extract per minute). A standard curve was generated to determine the relationship between fluorescence intensity and NADPH concentration.

Citrate synthase activity was quantified in a Beckman DU-700 spectrophotometer by measuring the reduction of NAD at 30 °C according to a procedure modified from that of Reichmann et al. (1983). A 50 µl sample of a 12.82 µg ml^{-1} muscle suspension in extraction buffer was added to 445 µl of citrate synthase reaction buffer in a quartz spectrophotometer cuvette. The reaction was started by adding 5 µl of a 30 mmol l^{-1} acetyl-CoA solution. The production of NADH was monitored at a wavelength of 340 nm for 10 min, and the slope of the absorbance curve was used to determine the activity of citrate synthase (expressed as µmoles of malate converted to citrate per gram of muscle extract per minute).

Morphology

We harvested the heart, liver, lungs, spleen, proventriculus, small intestine, large intestine, cecum and reproductive organs (testes in males; ovaries and oviduct in females) of each bird. We did not sample the kidneys because they were difficult to remove consistently without damage. We also did not sample the gizzard because we had trouble successfully removing the contents. Organs were trimmed of fat and connective tissue, emptied of contents (gut and oviduct), washed in physiological saline, blotted dry and weighed (to ±0.1 mg). They were then dried to constant mass at 60 °C and reweighed.

The entire right pectoralis muscle complex was carefully dissected from the skeleton and processed as described above. We removed, skinned and weighed the entire right leg and its musculature, detached the muscle and tendons (immediately proximal to the tibial joint), and weighed and processed these parts as described above. Nearly all the tissue from both pectoralis and leg muscle was recovered (we estimate that less than 1 % remained attached to the skeleton), and we were careful to treat each bird identically.

Statistical analyses

Because our data set included a substantial mass range and avian metabolic rate is a power function of mass (Lasiewski and Dawson, 1967), we $\log_{10}$-transformed mass and $\dot{V}_{O_2}$ to linearize these variables prior to most analyses. Analyses were performed using Statistica/Mac software (StatSoft, Inc.) and the SAS system software for personal computers, version 6.12 (SAS Institute, 1987). Significance was accepted at $P<0.05$, and results are expressed as mean ± S.E.M. unless noted otherwise.

We applied a sequential Bonferroni procedure to correct for Type I errors in multiple simultaneous tests (Rice, 1989).

Since the relationships between body mass, metabolic rate and morphology frequently differed between the sexes, we used separate equations for males and females where appropriate. To facilitate certain comparisons between males and females, we used an analysis of covariance (ANCOVA) and calculated values adjusted to a common mass (1075 g, the mean mass of all birds combined) using a least mean squares method. In reporting results from either analysis of variance (ANOVA) or ANCOVA, we report F values, with treatment and error degrees of freedom as subscripts, and P values.

All organ mass comparisons were corrected for the effects of body size. The method of correction depended on whether organs scaled isometrically or allometrically with body mass. We regressed wet and dry organ mass as a percentage of body mass against log-transformed body mass. If the slope was not significantly different from zero, we assumed that the relationship was isometric (i.e. that fractional organ mass is the same regardless of body mass). If the slope was significantly different from zero, we assumed that the relationship was allometric. For isometric organs, we generated correlations with metabolic rate using organ mass as percentage of fresh body mass. For allometric organs, we obtained body mass residuals of organ size and used them in analyses by plotting them against body mass residuals of either BMR or $\dot{V}_{O_2max}$.

To correct for the possible influence of body size on social behavior, we used ANCOVA with mass as covariate for comparisons between social ranks and for tests of the relationships between social rank and metabolic and morphological parameters.

Results

Metabolic rates and body mass

Data were obtained from 36 females and 36 males ranging in mass from 775 to 1570 g (954±15 g and 1197±25 g, respectively). There was a highly significant relationship between body mass and both BMR and $\dot{V}_{O_2max}$ for both sexes (P<0.003 in all cases; Fig. 1A). Substantial variance was present in both BMR and $\dot{V}_{O_2max}$ (Fig. 1B). Females had a significantly higher BMR than males (10.7±0.3 ml O_2 min^{-1} compared with 9.0±0.3 ml O_2 min^{-1}; $F_{1,70}$=15.3, P=0.0002, ANCOVA), while the converse was true for $\dot{V}_{O_2max}$ (76.0±2.7 ml O_2 min^{-1} compared with 115.5±2.8 ml O_2 min^{-1}; $F_{1,71}$=88.4, P<0.0001, ANCOVA). Accordingly, the absolute scope was 72 % larger for males (females 0.057±0.001 ml O_2 min^{-1} g^{-1}, males 0.098±0.002 ml O_2 min^{-1} g^{-1}; $F_{1,71}$=225.4, P<0.0001; Fig. 2A).

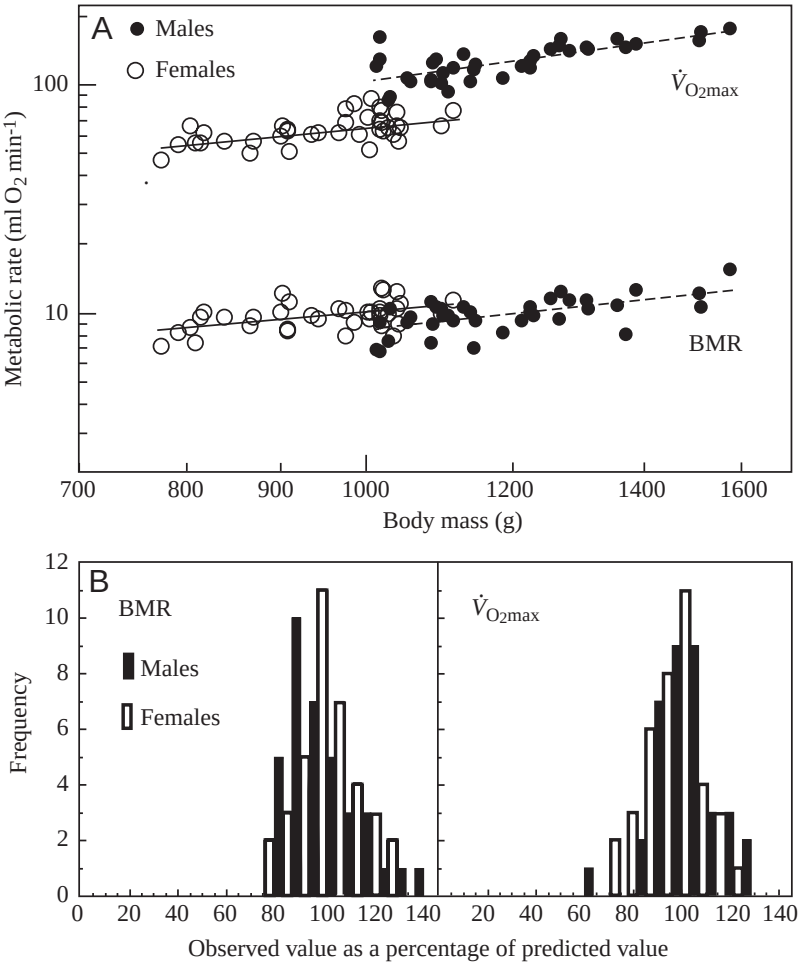

Fig. 1. (A) Allometry of basal metabolic rate (BMR) and maximal aerobic metabolic rate ($\dot{V}_{O_2max}$) for male and female red junglefowl. Both BMR and $\dot{V}_{O_2max}$ show significant gender differences. Equations for $\dot{V}_{O_2max}$ are: females, $\dot{V}_{O_2max}=0.177m^{0.86}$, r^2=0.35; males, $\dot{V}_{O_2max}=0.0449m^{1.12}$, r^2=0.53. Equations for BMR are: females, $BMR=0.0963m^{0.67}$, r^2=0.24; males, $BMR=0.0136m^{0.93}$, r^2=0.39, where m is mass. There are no differences in slope between the sexes for either BMR (male slope 0.93±0.2, female slope 0.67±0.21, P>0.05) or $\dot{V}_{O_2max}$ (male slope 1.21±0.18, female slope 0.87±0.20, P>0.05). (B) Variance in BMR and $\dot{V}_{O_2max}$ expressed as percentage difference from values predicted from mass allometry.

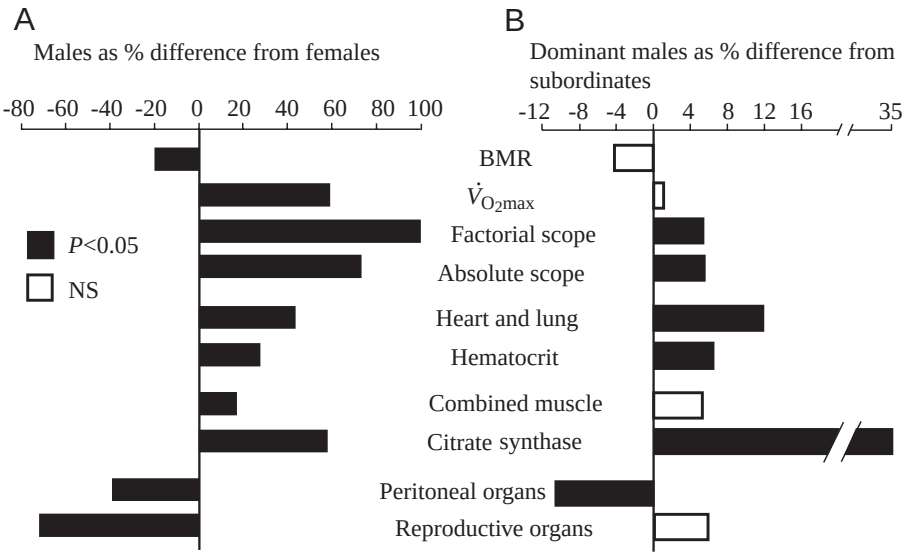

Fig. 2. Comparison of metabolic rates, organ masses, hematocrit, citrate synthase activity and factorial and absolute aerobic scope between (A) male and female red junglefowl and (B) dominant and subordinate males. BMR, basal metabolic rate; $\dot{V}_{O_2max}$, maximal aerobic metabolic rate; NS, not significant.

The factorial scope of males averaged 97% higher than that of females (males 13.1±0.5, females 6.6±0.2; $F_{1,70}$=74.82, P<0.0001, ANOVA).

Organ size and water content

The mean water content of the sampled muscles and organs was slightly higher in males than in females (74.7% compared with 72.4% respectively; $F_{1,70}$=28.3, P<0.0001, ANOVA). The organs that accounted for this difference were the cecum, liver, heart, muscle and reproductive organs. Male organs averaged a 1–4% higher water content than females organs. However, testes had a much greater water content than oviducts (86.1% compared with 72.4% respectively).

Because our qualitative conclusions were very similar for

Table 1. *Mean values for morphological and performance variables and the ratio of male to female values*

Grouping	Variable	Male mean	Male CV	Female mean	Female CV	Ratio	*F*	*P*
Body mass		1196.8	12.5	953.6	9.5	1.26	71.7	<0.0001*
Peritoneal	Proventriculus	0.064	16.6	0.099	20.2	0.64	89.5	<0.0001*
	Small intestine	0.236	17.9	0.438	15.1	0.54	245.3	<0.0001*
	Cecum	0.054	17.6	0.085	15.0	0.63	138.6	<0.0001*
	Large intestine	0.025	21.7	0.032	18.3	0.76	32.0	<0.0001*
	Spleen	0.019	29.4	0.017	12.0	1.07	0.8	<0.382
	Liver	0.358	14.0	0.542	11.7	0.66	189.9	<0.0001*
Cardiovascular	Heart (g)	0.136	18.0	0.084	12.0	1.62	147.1	<0.0001*
	Lung (g)	0.115	16.3	0.092	17.5	1.25	31.9	<0.0001*
	Hematocrit (%)	55.1	7.5	40.5	9.3	1.36	248.7	<0.0001*
Muscle	Leg muscle	4.818	11.6	3.736	7.5	1.29	109.7	<0.0001*
	Pectoralis muscle	3.782	11.2	3.676	8.6	1.03	1.5	<0.2278
Performance	BMR	0.0084	13.5	0.0103	12.8	0.81	45.8	<0.0001*
	$\dot{V}_{O_2max}$	0.1068	13.4	0.0676	12.0	1.58	207.0	<0.0001*
Reproductive	oviduct or testes	0.186	28.1	0.646	21.1	0.29	221.4	<0.0001*
Grouped totals	Muscle mass	8.599	10.5	7.410	6.6	1.16	51.5	<0.0001*
	Gut	0.376	15.2	0.657	13.0	0.57	265.8	<0.0001*
	Peritoneal	0.751	11.9	1.217	10.2	0.62	329.1	<0.0001*

Morphological variables are expressed as an absolute mass (body mass), or as dry mass as a percentage of fresh body mass.

Hematocrit is expressed as a percentage of blood volume. Basal metabolic rate (BMR) and maximal aerobic metabolic rate ($\dot{V}_{O_2max}$) are expressed in mass-specific units (ml O_2 g^{-1} body mass min^{-1}).

Asterisks (*) indicate significant differences between sexes after sequential Bonferroni corrections.

Values are means for 36 males and 36 females (N=72).

CV, coefficient of variation.

both wet and dry organs, we present only the results for dry masses and for dry organ mass as a percentage of body mass. While each sex showed considerable individual variability in organ masses, there were clearly discernible differences between males and females (Table 1; Fig. 2A).

Female intestinal tracts (proventriculus + small intestine + large intestine + cecae) were 75 % heavier than those of males ($F_{1,70}$=265.8, P<0.0001, ANOVA). This was true for the combined intestinal tract mass and for each component independently. Liver mass was 51 % larger in females than in males ($F_{1,72}$=189.9, P<0.0001, ANOVA), and female reproductive organs (oviducts) were 350 % heavier than male reproductive organs (testes). Even if reproductive organs are excluded, the combined mass of peritoneal organs (intestinal tract, liver and spleen) was 61 % larger in females than in males.

Organs of oxygen uptake and transport (hearts and lungs) of males were 43 % larger than those of females ($F_{1,72}$=145.8, P<0.0001, ANOVA). Male leg muscles were 29 % heavier than female leg muscles. There were no statistically significant differences in pectoralis muscle mass between the sexes, but total muscle mass (legs and pectoralis combined) was 16 % larger in males ($F_{1,72}$=51.5, P<0.0001, ANOVA).

Enzyme activity

We examined both mass-specific activity (per gram muscle mass) and whole-muscle activity (total activity for the entire muscle) for pectoralis and leg muscles (Table 2). Because whole-muscle activity is correlated with body mass, we used ANCOVA with body mass as a covariate for comparisons involving this parameter. However, we found no relationship between body mass and mass-specific activity for either enzyme in either muscle.

There was no difference between males and females in either mass-specific or whole-muscle hexokinase activity in pectoralis muscle. Females had a 30 % greater mass-specific hexokinase activity in leg muscle than males ($F_{1,64}$=12.02, P=0.0009, ANOVA), but there were no differences in whole-muscle leg hexokinase activity between the sexes.

In pectoralis muscle, the mass-specific citrate synthase activity of males was 17 % greater than that of females ($F_{1,66}$=6.8, P<0.0114, ANOVA) but there was no difference in whole-muscle activity. Mass-specific citrate synthase activity in leg muscle was 77 % greater in males than in females ($F_{1,65}$=57.6, P<0.0001, ANOVA), and whole-muscle leg citrate synthase activity was 75 % greater in males ($F_{1,64}$=15.9, P=0.0002, ANCOVA).

Since $\dot{V}_{O_2max}$ is a characteristic of the intact animal, it is reasonable to assume that it would be related to the combined and averaged enzyme activity of the two major muscle groups supporting locomotion, the leg and pectoralis complexes.

Table 2. *Mean mass-specific and total muscle enzyme activities for red junglefowl for (A) males and females and (B) dominant and subordinate males*

A Male/female differences

Muscle	Enzyme	Males	Females	*P* value
Pectoralis	Hexokinase (activity per gram muscle)	4.83±0.20	4.68±0.18	0.5629
	Total hexokinase (activity per muscle)	188.83±9.19	182.36±7.99	0.647
	Citrate synthase (activity per gram muscle)	3.78±0.16	3.22±0.15	0.0114*
	Total citrate synthase (activity per muscle)	146.37±10.12	133.31±8.99	0.407
Leg	Hexokinase (activity per gram muscle)	5.24±0.33	6.78±0.30	0.0009*
	Total hexokinase (activity per muscle)	266.81±17.83	266.28±15.78	0.9848
	Citrate synthase (activity per gram muscle)	7.90±0.34	4.45±0.30	0.0001*
	Total citrate synthase (activity per muscle)	388.59±27.08	222.70±23.51	0.0002*

B Status differences within males

Muscle	Enzyme	Dominants	Subordinates	*P* value
Pectoralis	Hexokinase (activity per gram muscle)	4.60±0.28	5.03±0.26	0.2559
	Total hexokinase (activity per muscle)	211.75±12.47	215.43±11.65	0.8316
	Citrate synthase (activity per gram muscle)	4.07±0.26	3.52±0.26	0.1538
	Total citrate synthase (activity per muscle)	185.46±14.57	155.77±14.10	0.1559
Leg	Hexokinase (activity per gram muscle)	4.42±0.44	5.96±0.41	0.0158*
	Total hexokinase (activity per muscle)	266.13±23.55	321.88±22.03	0.0954
	Citrate synthase (activity per gram muscle)	9.13±0.61	6.82±0.57	0.0098*
	Total citrate synthase (activity per muscle)	556.55±36.66	391.40±34.29	0.0028*

Values are means ± S.E.M. (N=74).
Asterisks indicate statistical significance using a sequential Bonferroni correction.
For hexokinase, the units of activity are μmoles of glucose converted to 6-P-gluconolactone per minute.
For citrate synthase, the units are μmoles of malate converted to citrate per minute.

Table 3. *Summary of multiple regressions for aerobic metabolic rate of red junglefowl*

	Males				Females			
Independent variables	$\dot{V}_{O_2max}$	Partial r^2	BMR	Partial r^2	$\dot{V}_{O_2max}$	Partial r^2	BMR	Partial r^2
Intercept	2.179	–	1.182	–	1.511	–	1.059	–
Hematocrit	0.006	0.073	–	–	0.005	0.156	–	–
Heart	2.398	0.422	–	–	–	–	–	–
Lung	–	–	–1.265	0.070	–	–	–	–
Spleen	–	–	–	–	–	–	–4.313	0.136
Proventriculus	–	–	–3.237	0.197	–	–	–	–
Small intestine	–	–	1.826	0.194	–	–	–	–
Cecum	–1.673	0.034	–2.516	0.054	–	–	–	–
Large intestine	–	–	–5.384	0.100	2.856	0.070	–	–
r^2	0.529		0.615		0.225		0.136	
P	0.0001		0.0001		0.0149		0.0293	

BMR, basal metabolic rate; $\dot{V}_{O_2max}$ maximal aerobic metabolic rate.

Accordingly, we obtained a mean activity value using the following formula: mean activity = (whole-muscle pectoralis activity + whole-muscle leg activity)/(leg mass + pectoralis mass). Mean citrate synthase activity was 57% greater in males than in females ($F_{1,60}$=62.9 P<0.0001, ANOVA). There were no significant gender differences in mean hexokinase activity.

Correlations between morphological, enzyme and metabolic variables

Because males were significantly larger than females and had different relationships between mass and $\dot{V}_{O_2}$ (Fig. 1A), we analyzed males and females separately.

In females, the only organ mass significantly correlated with BMR was spleen (Table 3), which explained 14% of the variance in BMR (P=0.025). In males 62% of the variance in BMR was explained by the masses of the small intestine, proventriculus, large intestine, lung and cecum (P<0.0001).

There were significant correlations between organ mass and $\dot{V}_{O_2max}$ for both males and females, but the particular organs differed between the sexes. In females, $\dot{V}_{O_2max}$ was positively correlated with large intestine mass (r=0.389, P=0.017) and hematocrit (r=0.408, P=0.012). In a multiple regression including hematocrit and all organ masses, hematocrit explained 16% of the variation in $\dot{V}_{O_2max}$ (P<0.0001) and large intestine mass explained an additional 7% (total explained variance was 23%, P=0.015; Table 4). Full correlation matrices are given in Table 5.

For males, heart (r=0.546, P=0.0006) and pectoralis muscle (r=0.451, P=0.006) mass were positively correlated with $\dot{V}_{O_2max}$, and cecum mass was negatively correlated with $\dot{V}_{O_2max}$ (r=–0.376, P=0.026). The total variance in $\dot{V}_{O_2max}$ explained by these organs was 53% (P<0.0001; Table 4). There were no significant correlations between reproductive organ mass and metabolic rates for either sex.

There was a significant correlation in males between pectoralis mass-specific citrate synthase activity and mass-specific $\dot{V}_{O_2max}$ (r=0.443, P=0.0125). However, there were no other significant correlations between enzyme activity of a single muscle and $\dot{V}_{O_2max}$.

BMR was not correlated with either mean hexokinase activity or mean citrate synthase activity for either sex. In contrast, $\dot{V}_{O_2max}$ was positively correlated with mean citrate synthase activity (r=0.72, P<0.0001) for both sexes combined, but this correlation is obviously driven by a positive correlation (r=0.47, P=0.0143) within males (Fig. 3). Within females, the relationship was not significant (r=–0.02, P=0.8985).

Correlations between BMR, $\dot{V}_{O_2max}$ and aerobic scope

After correction for body mass, we found no correlation between $\dot{V}_{O_2max}$ and BMR for either sex.

A priori, inter-individual variation in factorial scope is equally likely to result from variation in BMR or in $\dot{V}_{O_2max}$.

Table 4. *Summary of correlations between metabolic measurements and organ mass for red junglefowl*

Metabolic parameter	Sex	Variable	r	P value
BMR	Males	Small intestine	0.444	0.0066‡
	Females	Spleen	–0.368	0.0250‡
$\dot{V}_{O_2max}$	Males	Cecum	–0.376	0.0262
		Heart*	0.546	0.0006‡
		Pectoralis muscle	0.451	0.006‡
		Leg muscle*	0.321	0.056
		Total muscle mass*	0.388	0.0193‡
	Females	Large intestine	0.389	0.0173‡
		Hematocrit	0.408	0.0122‡

Asterisks indicate allometric relationships with body mass (analyses performed with mass residuals); double daggers indicate statistical significance by sequential Bonferroni correction.

See Table 5 for full correlation matrices.

BMR, basal metabolic rate; $\dot{V}_{O_2max}$ maximal aerobic metabolic rate.

Table 5. *Correlation matrix for basal metabolic rate, $\dot{V}_{O_2max}$, organ masses and hematocrit for females (below and to the left of the diagonal) and males (above and to the right of the diagonal)*

	Proventriculus	Small intestine	Cecum	Large intestine	Liver	Spleen	Heart	Lung	Pectoralis muscle	Leg muscle	Reproductive organs	HCT	BMR	$\dot{V}_{O_2max}$
Proventriculus		0.7476	0.5920	0.2476	0.2997	–0.0074	**0.1840**	–0.0178	0.0439	**0.4989**	0.0985	–0.1673	0.0377	–0.0951
		0.0001	0.0002	0.1454	0.0757	0.9658	**0.2827**	0.9178	0.7995	**0.002**	0.5678	0.3294	0.8270	0.5813
Small intestine	0.4028		0.6064	0.1775	0.3090	0.1771	**0.0530**	–0.0897	0.0394	**0.3213**	0.0525	–0.3281	0.4444	0.0423
	0.0122		0.0001	0.3004	0.0667	0.3015	**0.7592**	0.6029	0.8194	**0.056**	0.7611	0.0508	0.0066	0.8066
Cecum	0.17791	0.4983		0.1694	0.3248	0.0951	**–0.1086**	–0.1304	–0.3597	**0.0152**	0.1554	–0.1123	–0.0102	–0.3757
	0.2852	0.0015		0.3305	0.0569	0.5868	**0.5345**	0.4554	0.0338	**0.9309**	0.3726	0.5207	0.9536	0.0262
Large intestine	0.0340	0.3367	0.3727		0.1546	–0.1057	**0.0127**	–0.1989	–0.0996	**0.1199**	–0.0398	–0.0907	–0.2991	–0.2749
	0.8418	0.0416	0.0231		0.3681	0.5397	**0.9414**	0.2448	0.5635	**0.4861**	0.8180	0.5987	0.0764	0.1046
Liver	0.2762	0.2370	0.4634	0.1321		0.4792	**–0.0964**	–0.0857	–0.2330	**–0.0388**	–0.1444	–0.2771	0.1530	–0.3005
	0.0933	0.1520	0.0034	0.4357		0.0031	**0.5758**	0.6194	0.1714	**0.8222**	0.4008	0.1018	0.3730	0.0750
Spleen	0.2104	–0.0371	0.1192	–0.0910	0.4171		**–0.0911**	0.0098	0.0575	**0.0491**	0.0830	–0.2968	0.2828	–0.0011
	0.2048	0.8250	0.4761	0.5922	0.0092		**0.6194**	0.9550	0.7390	**0.7761**	0.6304	0.0788	0.0947	0.9948
Heart	0.4522	0.2388	0.3871	0.2129	0.3347	0.0961		**0.1067**	**0.2692**	**0.3718**	**0.0263**	**0.4174**	**0.0649**	**0.5457**
	0.0044	0.1487	0.0163	0.2059	0.0400	0.5659		**0.5357**	**0.1124**	**0.0255**	**0.8788**	**0.0113**	**0.707**	**0.0006**
Lung	–0.1302	0.0511	0.2795	0.0791	0.1514	–0.0006	0.1899		0.3179	**0.3929**	0.0491	–0.1673	–0.2676	0.0772
	0.4360	0.7604	0.0892	0.6416	0.3643	0.9973	0.2536		0.0589	**0.0178**	0.7760	0.3294	0.1146	0.6546
Pectoralis muscle	–0.1263	–0.2602	–0.3280	0.0162	–0.4056	–0.2175	–0.3260	–0.1915		**0.5343**	**0.2069**	**–0.116**	**–0.0109**	**0.4509**
	0.4498	0.1146	0.0444	0.9241	0.0115	0.1896	0.0458	0.2493		**0.0008**	**0.2259**	**0.5005**	**0.9499**	**0.0058**
Leg muscle	–0.1553	–0.3279	–0.2644	–0.1882	0.0860	0.2547	–0.2888	0.2416	0.2684		0.1859	–0.2116	0.2523	0.5026
	0.3519	0.0445	0.1087	0.2648	0.6078	0.1227	0.0786	0.1439	0.1033		0.2776	0.2154	0.1377	0.0018
Reproductive organs	0.1709	0.1792	–0.0759	0.0892	–0.2214	–0.4113	–0.0212	–0.0654	0.1927	–0.2113		0.2532	–0.0045	–0.0352
	0.3050	0.2817	0.6504	0.5995	0.1816	0.0103	0.8996	0.6965	0.2465	0.2029		0.1362	0.9793	0.8384
HCT	–0.1716	0.1069	0.3195	0.3095	–0.1171	–0.3279	0.0561	0.2569	0.1245	0.1214	–0.3595		–0.1894	–0.0271
	0.3099	0.5288	0.0539	0.0662	0.4902	0.0476	0.7418	0.1248	0.4628	0.4743	0.0288		0.2686	0.8752
BMR	0.0678	0.1272	–0.0128	0.1788	–0.2022	–0.3680	–0.0430	0.0396	–0.0824	–0.2910	0.2722	0.2852		**–0.330**
	0.6902	0.4532	0.9401	0.2968	0.2300	0.0250	0.8005	0.8162	0.6278	0.0806	0.1032	0.0918		**0.0494**
$\dot{V}_{O_2max}$	–0.0765	0.1109	0.2708	0.3890	–0.0850	–0.2826	0.0536	0.2211	0.1228	–0.1250	0.1276	0.4077	**–0.04634**	
	0.6478	0.5074	0.1001	0.0173	0.6118	0.0856	0.7495	0.1823	0.4628	0.4547	0.4453	0.0122	**0.7854**	

Correlations in bold type are those from allometric analyses, while the values in plain type are from isometric analyses.
The upper value for each cell is the correlation coefficient (r), and the lower value is the P value.
BMR, basal metabolic rate; $\dot{V}_{O_2max}$ maximal aerobic metabolic rate; HCT, hematocrit.

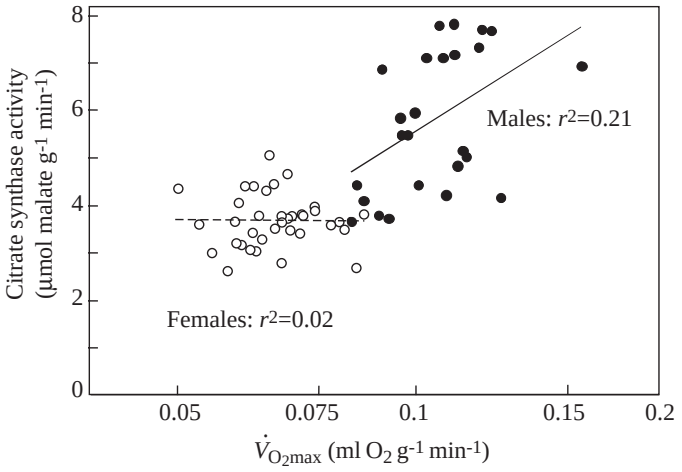

Fig. 3. Relationship between maximum aerobic metabolic rate ($\dot{V}_{O_2max}$) and mean citrate synthase activity (averaged over the leg and pectoralis muscles) for male and female red junglefowl. The relationship for females was not significant.

There was a negative correlation between absolute scope and BMR in both males (r=–0.77, P<0.0001) and females (r=–0.67, P<0.0001). There also was a negative correlation between factorial scope and BMR in both sexes (males, r=–0.91, P<0.0001; females, r=–0.87, P<0.0001). Unsurprisingly, correlations between $\dot{V}_{O_2max}$ and absolute scope were strongly positive for both sexes (males r=0.71, P<0.0001; females, r=64, P<0.0001), as were correlations between $\dot{V}_{O_2max}$ and factorial scope (males, r=0.48, P=0.0026; females, r=37, P=0.0223).

Social status

There were no statistically significant effects of any enzymatic, metabolic or morphological variable on the social status of females.

The social status of males was unrelated to $\dot{V}_{O_2max}$ and marginally related to BMR ($F_{1,33}$=4.1, P=0.052, ANCOVA; not significant). Thus, metabolic rate *per se* does not appear to determine social dominance and *vice versa*. However, dominant males had a slightly higher $\dot{V}_{O_2max}$ and a slightly lower BMR than subordinate males (neither difference was significant). As a result, both the absolute scope ($F_{1,34}$=5.2, P=0.029) and the factorial scope ($F_{1,34}$=4.9, P=0.034) of dominant males were significantly higher than those of subordinates.

Dominant males had larger hearts and lungs (combined $F_{1,33}$=14.2, P=0.0006, ANCOVA; Fig. 2B; heart alone $F_{1,33}$=6.8, P=0.014, ANCOVA; lungs alone $F_{1,33}$=5.6, P=0.024, ANCOVA) and higher hematocrit ($F_{1,33}$=7.8, P=0.009, ANCOVA) than subordinate males. Subordinate males had larger peritoneal organs (gut plus liver and spleen; $F_{1,32}$=8.1, P=0.008, ANCOVA), particularly liver ($F_{1,33}$=5.7, P=0.023, ANCOVA) and spleen ($F_{1,33}$=7.0, P=0.013, ANCOVA).

We found no significant differences in male pectoralis hexokinase activity related to social status, and there were no significant differences between dominant and subordinate males in pectoralis citrate synthase activity. Subordinate males had 35 % higher mass-specific leg hexokinase activity than dominant males ($F_{1,28}$=6.6, P=0.0158, ANOVA) but not a larger total leg hexokinase activity. Dominant males had a 34 % greater mass-specific leg citrate synthase activity than subordinates ($F_{1,28}$=7.7, P=0.0098, ANOVA) and a 42 % greater whole-muscle leg citrate synthase activity than subordinates ($F_{1,27}$=10.8, P=0.0028, ANCOVA). Mean citrate synthase activity was positively correlated with aerobic capacity in dominant males (r=0.65, P=0.023), but not in subordinates. No other regressions of enzyme activity were significant, either for mean values or for activity within each muscle type, after Bonferroni correction.

Discussion

We designed this study and analyzed our results in the context of four questions. (1) Do dominant male red junglefowl have higher aerobic capacities than subordinate males, and do they differ in organ properties? (2) Is aerobic capacity limited by the size of an individual's central organs (digestive tract, liver, heart, etc.), its peripheral effector organs (muscle) or both? (3) Is there a correlation between BMR and organ mass (central, peripheral or both) or muscle enzyme activity? (4) Is there a correlation between BMR and aerobic capacity, as predicted by the aerobic capacity model of endothermy (Bennett and Ruben, 1979) and indirectly by the central limitation and symmorphosis models?

Social status and aerobic capacity

Because the determination of dominance in females does not involve intense fighting, it is not surprising that we found no correlation between maximal aerobic performance and social status in females (also shown by Chappell et al., 1999b). In contrast, aggressive interactions between male junglefowl often involve intense and sustained combat (a behavioral tendency exploited by humans in staged 'cockfights'), so a high capacity for sustainable power output should be important in winning fights. Accordingly, we predicted a positive correlation between aerobic capacity and dominance status (in a related study, male junglefowl with a high $\dot{V}_{O_2max}$ had significantly more mating success in staged courtship trials than males with a low aerobic capacity; Chappell et al., 1997b). Although the relationship between male dominance status and $\dot{V}_{O_2max}$ was not significant in our tests, we did find that dominant males had higher absolute and factorial scopes. This is logical because (assuming that BMR is a fixed cost in all situations) absolute scope is the power output available to support muscular exercise, such as during combat. However, two caveats must be considered. First, we studied social rank within simple dyads; it would be useful to examine relationships between scope and rank in a more complex hierarchy. Second, in our tests, the establishment of dominance usually occurred fairly quickly and without intense fighting.

Therefore, it seems unlikely that aerobic performance *per se* played a direct role in determining rank in our tests. However, it is conceivable that competing males used behavioral or morphological cues from opponents to assess their capacity for prolonged combat.

There were interesting differences in the apparent allocation to various organs in dominant and subordinate males (Fig. 2B). Dominant birds had larger organs associated with aerobic performance (heart and lungs), while subordinates had larger organs associated with digestion and assimilation (peritoneal organs). Dominant males did not have larger muscles than subordinates, but they did have greater mean citrate synthase activity, which indicates a greater capacity for producing ATP aerobically. One possible interpretation of the contrast in 'investment' in muscle and organ mass and function is that dominants and subordinates experience differential benefits from organs that support maximal aerobic output and those that support digestion and intermediate metabolism. We speculate that dominant males may gain the most by maintaining their existing combat ability, but subordinates might benefit more from rapid growth. In any case, dominant males possess several morphological and functional traits that seem intuitively likely to aid in increasing aerobic performance.

Organ mass and enzymatic limitations on aerobic capacity

We predicted that if aerobic capacity was limited by effector organs, the mass and enzyme capacities of skeletal muscles would show a positive correlation with $\dot{V}_{O_2max}$, but there would not be a similar correlation with central organs. We also predicted that if aerobic capacity was limited by the physiological capacity of central organs (the mass and function of the digestive organs or the heart and lungs), then the central organs would show a positive correlation with $\dot{V}_{O_2max}$, but muscle properties would not.

We found that the aerobic capacity of males is correlated with both central (heart) and peripheral (muscle) organ mass and with one index of peripheral organ function (citrate synthase activity). This result is most consistent with the concept that aerobic capacity is controlled by system-wide co-adjusted limitations, e.g. symmorphosis (Taylor and Weibel, 1981). Chappell et al. (1999a) came to a similar conclusion about the limits to aerobic capacity in house sparrows (*Passer domesticus*). In contrast, aerobic capacity in female junglefowl is most strongly correlated to the mass of the central organs (large intestine and hematocrit) and not to muscle mass. Other bird species also make differential investments in the size (and presumably function) of the central and peripheral organs. However, previous reports attribute organ size differences to migration or to diet changes, instead of the sexual dimorphism seen in red junglefowl. For example, Starck (1999) showed that Japanese quail (*Coturnix japonica*) change the size of the gizzard in response to digestive challenges. In several elegant studies of shorebirds, Piersma and colleagues demonstrated substantial changes in their flight motor and digestive organ masses during migration and refueling. Migrating red knots (*Calidris canutus*) undergo flight muscle growth and a reduction in gizzard size shortly before migratory flights (Piersma et al., 1993; Battley and Piersma, 1997). Similarly, bar-tailed godwits (*Limosa lapponica*) reduce their stomach size during migration but increase it at stop-over sites for refueling (Piersma et al., 1993).

In addition to these studies of organ size plasticity, there are several reports of correlations between organ size and energy metabolism. Chappell et al. (1999a) examined relationships between the organ mass and aerobic performance of juvenile and adult house sparrows. As in the present study, they found a positive correlation between $\dot{V}_{O_2max}$, pectoralis mass and heart muscle mass; however, unlike the results for junglefowl (where the relationship is significant for males only), the positive correlation occurred in sparrows of both sexes. The difference may be related to the very different aerobic demands on male and female junglefowl (which is a ground-dwelling species in which only males engage in sustained fighting and therefore need a high $\dot{V}_{O_2max}$), as opposed to similar metabolic demands (and $\dot{V}_{O_2max}$) in male and female house sparrows (both sexes spend a considerable time flying, an extremely demanding form of exercise).

Bech and Østnes (1999) examined correlations between aerobic capacity during cold exposure and organ size in European shags (*Phalacrocorax aristotelis*). Unlike adults, nestling shags show a positive correlation between intestinal length and aerobic capacity, but not between aerobic capacity and heart, kidney or muscle mass. The authors suggest that chicks allocate energy to the central 'energy-processing' organs rather than to the effector or 'energy-consuming' organs, as would be expected of the much more active adults. This is not surprising because chicks presumably need to emphasize growth over activity (Chappell et al., 1997a; Chappell and Bachman, 1998).

Our data extend these results by showing that an important functional molecular parameter of aerobic respiration at the cellular level, the activity of citrate synthase in muscle, is correlated with aerobic capacity. Overall, male junglefowl have higher-capacity oxygen-delivery systems (heart, lungs and hematocrit), a greater muscle mass (leg and pectoralis muscle), a greater aerobic enzyme activity in major muscle groups and a much higher $\dot{V}_{O_2max}$ than females. Our results suggest that females make a greater investment in their visceral and reproductive organs (perhaps to support the large energy and materials flux during egg production).

Correlations with BMR

There have been numerous studies based on interspecific comparisons that suggest a relationship between BMR and organ mass in birds. Daan et al. (1989, 1990) reported strong positive correlations between heart and kidney mass and BMR in a variety of bird species, suggesting that BMR is largely a function of central organ size. Piersma et al. (1996) showed a positive correlation between lean body mass and BMR for red knots (*Calidris canutus*) in both winter and summer. Winter BMR was high and correlated with the grouped lean masses of 10 organs and summer BMR was low and correlated with the

same lean masses. Weber and Piersma (1996) showed a strong correlation between BMR and fat-free tissues with high metabolic activity, such as the heart during spring, after the peak migratory body mass loss, in *Calidris canutus*. In house sparrows, Chappell et al. (1999b) showed a positive correlation between BMR and the masses of the gut, reproductive organs liver, kidney and pectoralis muscle. Bech and Østnes (1999) demonstrated that liver mass is correlated best with resting metabolic rates during development in growing European shags (*Phalacrocorax aristotelis*) and that large intestine length and liver mass are highly correlated with resting metabolic rate in adult birds.

Several authors have suggested that increases in the mass of the metabolic machinery required to increase metabolic output will come at a cost of increased maintenance expenditure for those organs (e.g. Kersten and Piersma, 1987; Daan et al., 1989; Weiner, 1993; Hammond and Diamond, 1992; Piersma et al., 1996). At the whole-animal level, such a cost might be expressed as elevated BMR. More specifically, if increases in peak metabolic output (either for short-term bouts of intense exercise or for longer-term measures such as daily energy expenditure) are necessarily accompanied by increased maintenance costs, then the correlation with BMR should be strongest for those organs most directly responsible for the metabolic maxima in question.

This prediction was not supported by our data. We did find correlations between organ mass and BMR, but these involved organs (especially reproductive and peritoneal organs in females) whose mass was not correlated to $\dot{V}_{O_2max}$. We suggest that BMR may well be related to the costs of maintaining the capacity for high metabolic output, but these costs are probably associated with sustainable output over long periods. Our experimental design emphasized short-term aerobic power production (over periods of a few minutes), and it is probably unreasonable to expect correlations between these $\dot{V}_{O_2max}$ data and the maintenance costs of organs whose regulatory effects occur over much longer periods. Also, unlike many studies cited previously, it should be noted that we did not measure fat-free organ mass, so we do not know the effect of lean tissue content (presumably the metabolically active component) on BMR.

Aerobic capacity hypothesis

The aerobic capacity model of the evolution of endothermy (Bennett and Ruben, 1979) predicts a positive correlation between BMR and aerobic capacity. We did not find such a relationship in red junglefowl, but we did find correlations between factorial scope and both BMR and $\dot{V}_{O_2max}$. The correlations were stronger (and negative) for BMR, suggesting that a decline in BMR is largely responsible for increased factorial scope. We also found correlations between absolute scope and both BMR and $\dot{V}_{O_2max}$. The slopes were positive for $\dot{V}_{O_2max}$ and negative for BMR. As in a previous study of house sparrows (Chappell et al., 1999b), these results suggest that increased aerobic performance – at least for brief bouts of intense exercise – does not come at a cost of elevated maintenance metabolic rate.

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References

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J. Exp. Biol.* **90**, 17–32.

Battley, P. F. and Piersma, T. (1997). Body composition of lesser knots (*Calidris canutus rogersi*) preparing for take-off on migration from Northern New Zealand. *Notornis* **44**, 137–150.

Bech, C. and Østnes, J. E. (1999). Influence of body composition on the metabolic rate of nestling European shags (*Palacrocorax aristotelis*). *J. Comp. Physiol.* B **169**, 263–270.

Bennett, A. F. (1997). Adaptation and the evolution of physiological characters. In *New Directions in Ecological Physiology* (ed. W. H. Dantzler), pp. 3–16. New York: Oxford University Press.

Bennett, A. F. and Ruben, J. A. (1979). Endothermy and activity in vertebrates. *Science* **206**, 649–654.

Chappell, M. A. and Bachman, G. C. (1995). Aerobic performance in Belding's ground squirrels (*Spermophilus beldingi*): Variance, ontogeny and the aerobic capacity model. *Physiol. Zool.* **68**, 421–442.

Chappell, M. A. and Bachman, G. C. (1998). The exercise capacity of house wren nestlings: begging chicks are not working as hard as they can. *Auk* **115**, 863–870.

Chappell, M. A., Bachman, G. C. and Hammond, K. A. (1997a). The heat increment of feeding in house wren chicks: magnitude, duration and substitution for thermostatic costs. *J. Comp. Physiol.* B **167**, 313–318.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999a). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269–2279.

Chappell, M. A. and Snyder, L. R. G. (1984). Biochemical and physiological correlates of deer mouse alpha-chain hemoglobin polymorphisms. *Proc. Natl. Acad. Sci. USA* **81**, 5484–5488.

Chappell, M. A., Zuk, M. and Johnsen, T. S. (1996). Repeatability of aerobic performance in red junglefowl: effects of ontogeny and nematode infection. *Funct. Ecol.* **10**, 578–585.

Chappell, M. A., Zuk, M. and Johnsen, T. S. (1999b). Aerobic performance does not affect social rank in female red jungle fowl. *Funct. Ecol.* **13**, 163–169.

Chappell, M. A., Zuk, M., Johnsen, T. S. and Kwan, T. H. (1997b). Mate choice and aerobic capacity in red junglefowl. *Behaviour* **134**, 511–529.

Daan, S., Masman, D. and Groenewold, A. (1990a). Avian basal metabolic rates: their association with body composition and energy expenditure in nature. *Am. J. Physiol.* **259**, R333–R340.

Daan, S., Masman, D., Strijkstra, A. M. and Kenagy, G. J. (1990). Daily energy turnover during reproduction in birds and mammals:

its relationship to basal metabolic rate. In *Congressus Internationalis Ornithologica*, vol. IV (ed. B. D. Bell, R. O. Cossee, J. E. C. Flux, B. D. Heather, R. A. Hitchmough, C. J. R. Robertson and M. J. Williams), pp. 1976–1987. Christchurch: New Zealand Ornithological Congress Trust Board.

Daan, S., Masman, D., Strijkstra, A. and Verhulst, S. (1989). Intraspecific allometry of basal metabolic rate: relations with body size, temperature, composition and circadian phase in the kestrel, *Falco tinnunculus*. *J. Biol. Rhythms* **4**, 267–283.

Else, P. L. and Hulbert, A. J. (1981). Comparison of the 'mammal machine' and the 'reptile machine': energy production. *Am. J. Physiol.* **240**, R3–R9.

Garland, T. (1984). Physiological correlates of locomotory performance in a lizard: an allometric approach. *Am. J. Physiol.* **247**, R806–R815.

Garland, T. and Bennett, A. F. (1990). Quantitative genetics of maximal oxygen consumption in a garter snake. *Am. J. Physiol.* **259**, R986–R992.

Garland, T. and Else, P. (1987). Seasonal, sexual and individual variation in endurance and activity metabolism in lizards. *Am. J. Physiol.* **259**, R986–R992.

Hammond, K. A. and Diamond, J. M. (1992). An experimental test for a ceiling on sustained metabolic rate in lactating mice. *Physiol. Zool.* **65**, 952–977.

Hammond, K. A., Konarzewski, M., Torres, R. and Diamond, J. (1994). Metabolic ceilings under a combination of peak energy demands. *Physiol. Zool.* **68**, 1479–1506.

Hulbert, A. J. and Else, P. (1981). Comparison of the 'mammal machine' and the 'reptile machine': energy use and thyroid activity. *Am. J. Physiol.* **241**, R350–R356.

Jayne, B. C. and Bennett, A. F. (1990). Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**, 1204–1229.

Kersten, M. and Piersma, T. (1987). High levels of energy expenditure in shore birds: Metabolic adaptations to an energetically expensive way of life. *Ardea* **75**, 175–187.

Konarzewski, M. and Diamond, J. (1995). Evolution of basal metabolic rate and organ masses in laboratory mice. *Evolution* **49**, 1239–1248.

Lasiewski, R. C. and Dawson, W. R. (1967). An examination of the relation between standard metabolic rate and body weight in birds. *Condor* **69**, 13–23.

Passonneau, J. V. and Lowry, O. H. (1993). *Enzymatic Analysis: A Practical Guide.* Totowa, NJ: Humana Press.

Peterson, C. C., Nagy, K. A. and Diamond, J. (1990). Sustained metabolic scope. *Proc. Natl. Acad. Sci. USA* **87**, 2324–2328.

Piersma, T., Bruinzeel, L., Drent, R., Kersten, M., Van der Meer, J. and Wiersma, P. (1996). Variability in basal metabolic rate of a long-distance migrant shorebird (red knot *Calidris canutus*) reflects shifts in organ sizes. *Physiol. Zool.* **69**, 191–217.

Piersma, T., Koolhaas, A. and Dekinga, A. (1993). Interactions between stomach structure and diet choice in shorebirds. *Auk* **110**, 552–564.

Reichmann, H., Srihari, T. and Pette, D. (1983). Ipsi- and contralateral fibre transformation by cross-reinnervation. A principle of symmetry. *Pflügers Arch.* **397**, 202–208.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Ricklefs, R. E., Konarzewski, M. and Daan, S. (1996). The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *Am. Nat.* **147**, 1047–1071.

SAS Institute (1987). *SAS/STAT Guide for Personal Computers. Version 6 Edition*. Cary, NC: SAS Institute Inc.

Starck, J. M. (1999). Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes of organ size in response to changes in dietary fiber content. *J. Exp. Biol.* **202**, 3171–3179.

Taylor, C. R. and Weibel, E. R. (1981). Design of the mammalian respiratory system. I. Problems and strategy. *Respir. Physiol.* **44**, 1–10.

Weber, T. P. and Piersma, T. (1996). Basal metabolic rate and the mass of tissues differing in metabolic scope: migration-related covariation between individual knots, *Calidris canutus. J. Avian Biol*. **27**, 215–224.

Weibel, E. R. (1984). *The Pathway for Oxygen: Structure and Function in the Mammalian Respiratory System*. Cambridge, MA: Harvard University Press.

Weibel, E. R., Taylor, C. R. and Hoppeler, H. (1991). The concept of symmorphosis: a testable hypothesis of structure–function relationship. *Proc. Natl. Acad. Sci. USA* **88**, 10357–10361.

Weiner, J. (1993). Physiological limits to sustainable energy budgets in birds and mammals: Ecological implications. *Trends Ecol. Evol.* **7**, 384–388.

Zuk, M., Kim, T., Robinson, S. I. and Johnsen, T. S. (1998). Parasites influence social rank and morphology, but not mate choice, in female red junglefowl, *Gallus gallus*. *Anim. Behav.* **56**, 493–499.

Zuk, M., Popma, S. L. and Johnsen, T. S. (1995). Male courtship displays, ornaments and female mate choice in captive red junglefowl. *Behaviour* **132**, 11–12.

Zuk, M., Thornhill, R. and Ligon, J. D. (1990). Parasites and mate choice in red junglefowl. *Am. Zool.* **30**, 232–244.