Repeatability of aerobic performance in Red Junglefowl: effects of ontogeny and nematode infection

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Summary

1. Repeatability is important in determining how traits are affected by selection, but it is largely unstudied for most physiological characters other than locomotory performance. We examined the repeatability of aerobic capacity, measured as maximum O_2 consumption ($\dot{V}_{O_{2max}}$), in Red Junglefowl, *Gallus gallus*. $\dot{V}_{O_{2max}}$ is an integrated index of metabolic performance that can be intuitively linked to fitness because it sets the upper limit to sustainable power output.

2. We used mass residuals to determine $\dot{V}_{O_{2max}}$ repeatability in adult birds, and across growth from 28-day-old chicks to reproductively mature adults. We measured $\dot{V}_{O_{2max}}$ during brief episodes of intense exercise in motorized running wheels. Minimal resting metabolism ($\dot{V}_{O_{2mr}}$) was measured in adults to provide an estimate of factorial aerobic scope ($\dot{V}_{O_{2max}}/\dot{V}_{O_{2mr}}$). We also examined the influences of sex and infection by a common intestinal nematode, *Ascaridia galli*, on $\dot{V}_{O_{2max}}$.

3. There were no gender differences in adult $\dot{V}_{O_{2mr}}$ or in the $\dot{V}_{O_{2max}}$ of chicks. However, the $\dot{V}_{O_{2max}}$ of adult males was considerably greater than that of adult females. Factorial aerobic scopes were 9·6 and 5·6 for adult males and females, respectively. Higher scope in males may be an adaptation to support intense, prolonged inter-male aggression. Infection with *A. galli* significantly depressed the mass and $\dot{V}_{O_{2max}}$ of chicks but had no effect on adults.

4. The $\dot{V}_{O_{2max}}$ of adult junglefowl was highly repeatable over periods up to 180 days. In contrast, we found no repeatability of $\dot{V}_{O_{2max}}$ across ontogeny for birds tested initially as chicks and again as adults. The lack of repeatability was not influenced by sex or infection status.

Key-words: Aerobic capacity, between-individual variation, exercise, phenotypic plasticity

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Introduction

Repeatability – the consistency over time of an individual's morphology or performance ranking within a population – is a critical factor determining how a trait can evolve under selection (Bennett 1987). Lack of repeatability greatly reduces the potential effects of selection. From this perspective, physiological ecologists have intensively studied the repeatability of locomotor traits such as sprint speed and endurance (Putnam & Bennett 1981; Tolley, Notter & Marlowe 1983; Garland 1985; Arnold & Bennett 1988; Djawdan & Garland 1988; Austin & Shaffer 1992). Locomotor performance traits are popular for repeatability studies because they are easy to measure quickly and harmlessly, and because they are probably important for survival. A number of studies have reported significant repeatability of sprint speed and endurance, even in wild populations and across considerable portions of life

span (Huey & Dunham 1987; Van Berkum *et al.* 1989; Shaffer, Austin & Huey 1991).

The repeatabilities of many other key physiological characters are poorly understood. Surprisingly little is known about repeatability of aerobic metabolism, even though this is one of the most intensively studied and ecologically important aspects of organismal physiology, particularly in vertebrate endotherms. Moreover, mechanistic and comparative physiologists routinely view aerobic metabolism as a Darwinian adaptation in response to selection by various ecological or environmental factors. The mechanisms for this putative evolutionary response are unclear, however, since metabolic rates in most species studied to date are highly plastic under exercise conditioning regimes, temperature acclimation, developmental maturation, infection by parasites, etc. Such plasticity may affect repeatability and confound any effects of selection. For example, conditioning or acclimation might produce changes in absolute aerobic perfor-

mance but leave relative performance rankings of individuals unaltered (resulting in high repeatability and high potential for evolutionary change under selection, provided that performance is heritable), or they might affect both absolute values and ranking (resulting in little or no repeatability and a correspondingly weak effect of selection).

We are aware of only four studies of the repeatability of vertebrate aerobic metabolism. All of these studies (Garter Snakes, *Thamnophis sirtalis,* Garland & Bennett 1990; Deer Mice, *Peromyscus maniculatus,* Hayes 1989a,b; Hayes, & Chappell 1990; Belding's Ground Squirrels, *Spermophilus beldingi,* Chappell, Bachman & Odell 1995) found significant repeatability over short intervals (hours or days) and in some cases over extended periods (months or years). However, there was no evidence of repeatability of aerobic performance across growth from juveniles to adults in the one species for which data are available (Belding's Ground Squirrels; Chappell *et al.* 1995) – in contrast to the findings for sprint speed in wild populations of lizards (Huey & Dunham 1987; Van Berkum *et al.* 1989).

Here we report results of repeatability studies on the maximal aerobic performance of a ground-dwelling bird, the Red Junglefowl *Gallus gallus* (Linnaeus). We discuss performance repeatability over intervals ranging from 2 h to 6 months in adult birds, and across 8 months of rapid growth from small chicks to reproductively mature adults. To our knowledge, this is the first examination of long-term repeatability of aerobic traits in a bird. We also discuss the influence of sex (there is considerable sexual dimorphism in morphology, behaviour and physiology in this species). Finally, we describe the effects of a common intestinal parasite, *Ascaridia galli* (Schrank), on aerobic performance and its repeatability. Although blood-borne parasites that attack erythrocytes (such as malaria) are known to reduce aerobic capacity (presumably with associated effects on behaviour and fitness; e.g. Schall, Bennett and Putnam 1982), the performance effects of more benign but often abundant parasites such as *A. galli* are little known.

Methods

ANIMALS AND TREATMENTS

Red Junglefowl were reared from eggs obtained from a captive flock maintained under semi-natural conditions (outdoor pens exposed to ambient weather conditions and photoperiod in Riverside, California; Zuk *et al.* 1990a; Zuk, Johnsen & MacLarty 1995). Birds were obtained from the feral flock at the San Diego Zoo in southern California in the late 1980s and have been reared in captivity using a minimum of approximately 50 sires and 50 dams since that time. No artificial selection for activity patterns, physiology or reproductive biology has occurred.

Adult performance and its repeatability were studied in a group of mature birds (>1-year-old at the time of initial measurements) in good health. To examine performance during ontogeny and the effects of *A. galli* infection we divided a cohort of newly hatched chicks into two treatment groups. One group was inoculated with *A. galli* (~100 embryonated eggs administered orally in saline every week between the ages of 1–4 weeks; Zuk *et al.* 1990a), and a second group served as controls (they received saline only or no treatment; there was no significant difference between mass or metabolism of the saline and no treatment groups). At the age of 6 weeks, chicks were moved from the brooders to outdoor pens, where they resided until tested as adults. Parasitized and unparasitized junglefowl were housed separately (birds normally acquire *A. galli* infection from pecking at ground contaminated with faeces). All individuals tested as both chicks and adults were killed after the completion of measurements and dissected to verify infection status (we use the term 'adult' for birds that had attained reproductive maturity as determined from size and secondary sexual characters).

METABOLIC MEASUREMENTS

We measured oxygen consumption ($\dot{V}_{O_2}$) using open-circuit respirometry. Mass flow controllers (Tylan or Applied Materials) supplied dry air at constant rates (±2%) under positive pressure. About 100 ml/min of chamber excurrent air was dried (Drierite), scrubbed of CO_2 (Ascarite), re-dried and flowed through the sensor of an oxygen analyser (Applied Electrochemistry S-3A/II). Instrument outputs were sampled by a Macintosh computer equipped with a National Instruments analogue-to-digital converter and custom graphical data-acquisition and analysis software. Multiple readings (60–100) were averaged for each recorded datum. With digital signal averaging, the resolution of the system was 0·0015–0·002% O_2. The worst-case cumulative error in $\dot{V}_{O_2}$ calculations was about ±5%. Repeatability of the equipment was greater than absolute accuracy, so the maximum cumulative error for comparisons between individuals (or for repeated measures on the same individual) was about 3%. We measured $\dot{V}_{O_2max}$ during the day (active phase) and minimum resting metabolism ($\dot{V}_{O_2mr}$) at night (inactive phase).

MINIMUM RESTING METABOLISM

$\dot{V}_{O_2mr}$ was measured in a 55-l acrylic chamber placed in a darkened environmental cabinet that maintained ambient temperature (T_a) at 30–32°C (within thermoneutrality for Red Junglefowl; unpublished data). Birds were fasted (but had access to water) for >8 h prior to measurements. Flow rates were 8 to 12 l min⁻¹ STP (standard temperature and pressure). Oxygen concentration, flow rate and T_a were recorded every

5–10 s for 2–3 h. We determined $\dot{V}_{O_2mr}$ with software that searched data files for the lowest $\dot{V}_{O_2}$ averaged over continuous 5-min intervals.

$\dot{V}_{O_2max}$ MEASUREMENTS

We measured $\dot{V}_{O_2max}$ in enclosed running wheels constructed of acrylic plastic and driven by variable-speed motors. Air was pumped through gas-tight axial bearings, and running tracks of thin carpet provided traction and injury protection. For chicks, we used a wheel with an inside diameter of 32 cm and width of 11 cm, yielding a circumference of 1 m and an internal volume of 9 l. Air flow rates were 5·51 min^{-1} STP. For adults, we used a larger wheel with an inside diameter of 76 cm and width of 42 cm, yielding a circumference of 2·4 m and an internal volume of 190 l. Flow rates for this wheel were 39–43 l min^{-1} STP. For both wheels, tread speeds ranged from 0·4 to about 2·5 m s^{-1} (in practice, few birds ran faster than 2 m s^{-1}). During runs, the computer recorded and displayed O_2 concentration, flow rate, and speed every 1·5 s.

Junglefowl were placed in the wheel and allowed 2–3 min to adjust while baseline O_2 concentration and 'resting' $\dot{V}_{O_2}$ were measured. The wheel was then started and maintained at its lowest speed until the bird adjusted to the movement and walked or ran to maintain position. This generally required 15–30 s. Speed was gradually increased in steps of 0·2–0·3 m s^{-1} while $\dot{V}_{O_2}$ was continuously monitored. We assumed $\dot{V}_{O_2max}$ had been attained when $\dot{V}_{O_2}$ did not increase with increasing speed, or when birds no longer maintained coordinated locomotion or position in the wheel (birds showed obvious signs of exhaustion at this point, but none suffered any injury). Complete measurements lasted 4–8 min in chicks (1·5–5 min of actual exercise) and 5–15 min in adults (2–11 min of actual exercise).

To compensate for mixing and resolve short-term changes in $\dot{V}_{O_2}$, we calculated 'instantaneous' $\dot{V}_{O_2}$ (Bartholomew, Vleck & Vleck 1981) using effective volumes of 8·2 l (small wheel) and 188 l (large wheel). Effective volume was obtained from washout curves resulting from near-instantaneous injections of oxygen-depleted air (Bartholomew *et al.* 1981). The $\dot{V}_{O_2max}$ was computed as the highest $\dot{V}_{O_2}$ averaged over continuous 1- and 2-min intervals. Although the 2-min averages were slightly lower than the 1-min averages (Hayes, Speakman & Racey 1992), there was no qualitative difference between the two intervals in the analyses described below; accordingly we restrict our discussion to the 1-min averages.

STATISTICS

Because metabolism is a power function of mass, we log$_{10}$-transformed mass and metabolism data prior to analysis. We used *t*-tests to assess differences in

mass, and covariance procedures to test for metabolic differences between groups while accounting for mass effects.

Repeatability is assessed from the relationship between initial and final measurements, but a mass correction was necessary because our data sets contained mass ranges of 2- to 40-fold and the mass of individuals often changed substantially between measurements. To compensate, we calculated repeatability from residuals of least-squares regressions of log$_{10}$-transformed mass and metabolism. A significant positive correlation between initial and final residuals demonstrates repeatability. This procedure tests the hypothesis that relative performance ranking for the initial measurement predicts relative performance ranking for the final measurement. Analyses were performed with Statistica/Mac software (StatSoft, Inc.). We applied a sequential Bonferroni procedure to correct for Type I errors in multiple simultaneous tests (Rice 1989). The significance level was $P = 0·05$.

Results

EFFECTS OF SEX ON AEROBIC PERFORMANCE AND SCOPE

Minimal metabolic rates of adults were not affected by sex ($F_{1,27} = 3·62$, $P = 0·07$; $n = 11$ males and 19 females) and there was no difference between $\dot{V}_{O_2max}$ of male and female chicks ($F_{1,49} = 0·45$, $P = 0·58$; $n = 19$ females and 33 males). However, we found large and significant differences between the $\dot{V}_{O_2max}$ of adult males and females (Fig. 1; $F_{1,143} = 171$, $P < 0·00001$; $n = 80$ males and 65 females). At standardized body masses, male $\dot{V}_{O_2max}$ was 54% higher than female $\dot{V}_{O_2max}$. Accordingly, separate regressions for each sex (Table 1) were used to compute residuals for analysis of performance repeatability of adults.

Mean mass was 1231 ± 180 g for adult males and 975 ± 138 g for adult females. For these masses, the factorial aerobic scope ($\dot{V}_{O_2max}/\dot{V}_{O_2mr}$) computed from
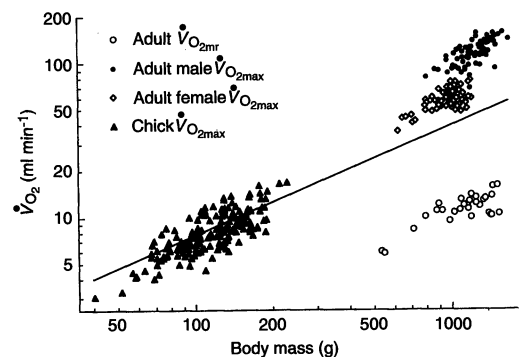

Fig. 1. Aerobic metabolism (minimum resting metabolism, $\dot{V}_{O_2mr}$; maximum oxygen consumption, $\dot{V}_{O_2max}$) as a function of body mass in Red Junglefowl. The regression line is for the $\dot{V}_{O_2max}$ of chicks not infected with the intestinal parasite *Ascaridia galli* (this parasite has no effect on adult $\dot{V}_{O_2}$).

Table 1. Minimum and maximum oxygen consumption ($\dot{V}_{O2mr}$ and $\dot{V}_{O2max}$; ml/min) of adult Red Junglefowl, and $\dot{V}_{O2max}$ of 4-week-old chicks. Adult males had significantly higher $\dot{V}_{O2max}$ than adult females, but sex did not affect the $\dot{V}_{O2max}$ of chicks. Infection with the nematode *Ascaridia galli* affected chick $\dot{V}_{O2max}$, but not that of adults

Adult $\dot{V}_{O2mr} = 0.144 \, mass^{0.621}$	$n = 30, \, F_{1,28} = 40.8, \, r^2 = 0.59, \, P < 0.00001$
Adult male $\dot{V}_{O2max} = 0.0736 \, mass^{1.033}$	$n = 80, \, F_{1,78} = 77, \, r^2 = 0.50, \, P < 0.00001$
Adult female $\dot{V}_{O2max} = 0.557 \, mass^{0.675}$	$n = 65, \, F_{1,63} = 47, \, r^2 = 0.43, \, P < 0.00001$
Infected chick $\dot{V}_{O2max} = 0.207 \, mass^{0.791}$	$n = 120, \, F_{1,118} = 198, \, r^2 = 0.63, \, P < 0.00001$
Control chick $\dot{V}_{O2max} = 0.322 \, mass^{0.689}$	$n = 60, \, F_{1,58} = 44, \, r^2 = 0.43, \, P < 0.0001$
Overall chick $\dot{V}_{O2max} = 0.190 \, mass^{0.786}$	$n = 180, \, F_{1,178} = 283, \, r^2 = 0.62, \, P < 0.00001$
Chick to adult female $\dot{V}_{O2max} = 0.102 \, mass^{0.919}$	$n = 125, \, F_{1,123} > 1000, \, r^2 = 0.96, \, P < 0.00001$
Chick to adult male $\dot{V}_{O2max} = 0.0418 \, mass^{1.108}$	$n = 140, \, F_{1,138} > 1000, \, r^2 = 0.97, \, P < 0.00001$

regressions of mass and minimal and maximal metabolism was 9·6 for males and 5·6 for females.

VARIANCE

We estimated variance as the ratio of observed $\dot{V}_{O2}$ to the $\dot{V}_{O2}$ predicted by regressions of $\dot{V}_{O2}$ and mass. Mean differences between observed and predicted values ranged from 10 to 15% in adult $\dot{V}_{O2mr}$ and in the $\dot{V}_{O2max}$ of both adults and chicks (Fig. 2).

EFFECTS OF *ASCARIDIA GALLI* INFECTION

We found no effects of *A. galli* infection on either the mass ($t = 0.13$, $P = 0.72$) or the $\dot{V}_{O2max}$ ($F_{1,47} = 0.12$, $P = 0.73$) of adult junglefowl ($n = 33$ males and 19 females of known infection status). Similarly, this nematode had no effect on adult $\dot{V}_{O2mr}$ ($P = 0.46$, $n = 15$ males and 5 females of known infection status).

Twenty-eight-day-old chicks inoculated with *A. galli* were significantly smaller than control chicks (111 ± 7.6 g, $n = 120$ versus 137.4 ± 9.9 g, $n = 60$, respectively; $P < 0.0001$). Also, ANCOVA revealed slight but significant differences between the $\dot{V}_{O2max}$ of inoculated and control chicks ($F_{2,176} = 4.6$,

Fig. 2. Variance in aerobic metabolism of Red Junglefowl. Variance is expressed as (observed value/predicted value); predicted values were calculated from regressions of $\dot{V}_{O2}$ against mass (Table 1). *N* is the number of measurements; $\bar{x}$ is the mean of the variance (expressed as absolute value of precentage differences from predicted values).

$P = 0.011$; Fig. 3; Table 1). Mass exponents did not differ significantly ($P = 0.50$), but inoculated chicks suffered a decrease of about 10% in maximal aerobic performance after accounting for mass differences.

REPEATABILITY OF $\dot{V}_{O2max}$

We obtained data on the repeatability of maximal aerobic performance in adult junglefowl from an initial cohort of 31 birds ranging in mass from 615 to 1697 g. Repeatability (*r*) was highly significant across measurement intervals ranging from 2 h to 180 days (Fig. 4; Table 2), with *r* values ranging from 0·93 to 0·51. Mass of these individuals changed very little between measurements (mean change 3·3%, maximum change 17·2%).

Repeatability of $\dot{V}_{O2max}$ during ontogeny was determined from an initial cohort of 180 chicks measured at age 28 ± 2 days, of which 52 were subsequently tested at reproductive maturity (age 8–9 months). These individuals were all the survivors of the initial cohort that were available for measurements (i.e. had not suffered injury or been used for other studies). Because of high variance in initial growth rates, chick masses ranged from 95 to 229 g (mean 139 ± 29 g). Thirty-four chicks were infected with *A. galli* (mean mass 135 ± 28 g) and 18 were uninfected (mean mass 146 ± 31 g). Variance in adult mass was also substantial (males, 962–1542 g, mean 1248 ± 165 g, $n = 33$; females, 793–1128 g, mean 973 ± 99 g, $n = 19$) but mass was not affected by infection. There was no correlation between chick and adult mass ($P = 0.89$ for females; $P = 0.68$ for males) in either infected or uninfected birds. Similarly, we found no $\dot{V}_{O2max}$ repeatability during ontogeny, and no indication that repeatability was influenced by either sex or *A. galli* infection (Table 3).

Discussion

MEASUREMENT PROTOCOLS

The reliability of protocols to measure the maximal aerobic metabolism of volant birds requires careful scrutiny, particularly for methods based on running
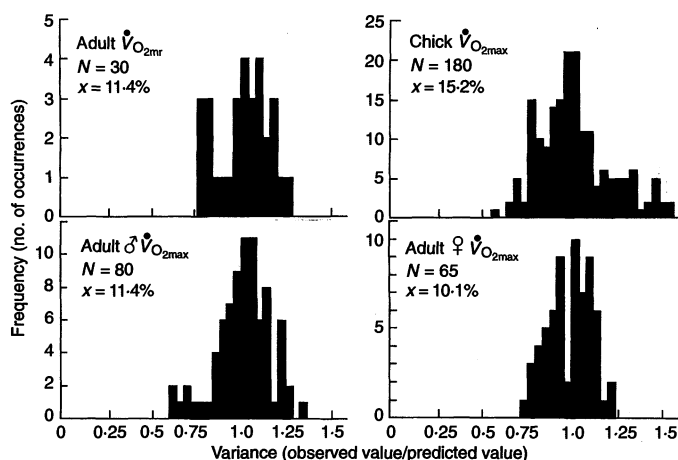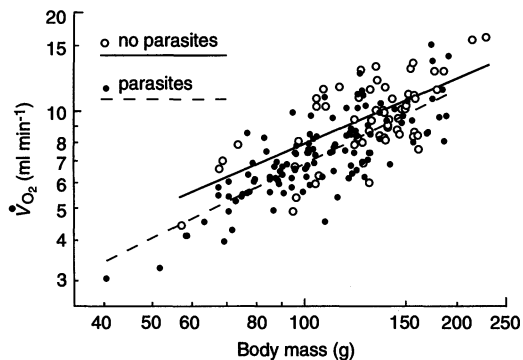
Fig. 3. The $\dot{V}_{O2max}$ of parasitized and unparasitized Red Junglefowl chicks as a function of mass. All birds were 28 ± 2-days old; mean mass was significantly smaller for infected chicks. Infected chicks had significantly lower $\dot{V}_{O2max}$ than uninfected chicks, but mass exponents did not differ significantly.
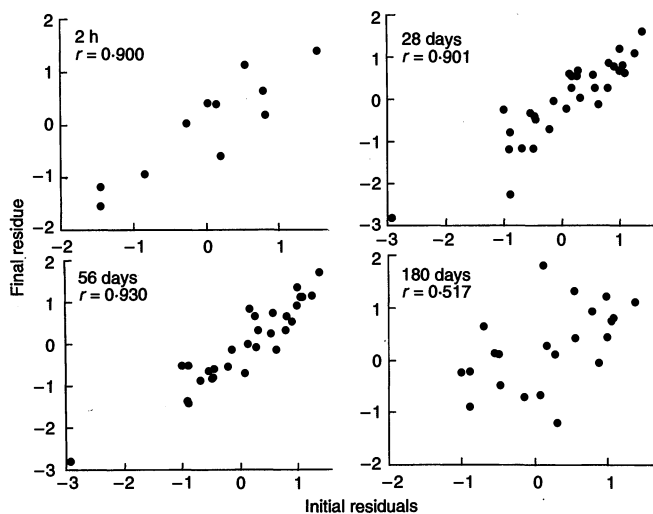

Fig. 4. Repeatability of adult $\dot{V}_{O2max}$ across four time intervals. Repeatability was estimated from the correlation of initial versus final residuals from regressions of $\log_{10} \dot{V}_{O2max}$ on $\log_{10}$ body mass. Repeatability is significant for all measurement intervals, but declines at the longest interval (180 days).

treadmill exercise (10–11 in rheas and domestic fowl; Taylor *et al.* 1971; Brackenbury & El-Sayed 1985). Third, all of the Red Junglefowl we tested (including chicks) flapped vigorously for 15–45 s at the beginning of exercise bouts. We detected no tendency for the highest $\dot{V}_{O2}$ to occur during this period (as opposed to the middle or end segments of exercise tests when birds were generally not flapping), indicating that recruitment of flight muscles did not substantially increase sustainable $\dot{V}_{O2}$. Finally, all individuals showed obvious behavioural evidence of exhaustion during exercise tests. These observations, together with the high repeatability of $\dot{V}_{O2max}$ in adults, make us confident that our protocol successfully elicited maximal aerobic performance, at least over relatively short measurement periods.

FACTORS AFFECTING AEROBIC CAPACITY

Both sex and infection by *Ascaridia galli* influenced $\dot{V}_{O2max}$, but their effects occurred at different life stages: *A. galli* infection decreased the $\dot{V}_{O2max}$ of chicks but did not affect the $\dot{V}_{O2max}$ of adults, while sex had no effect on chick $\dot{V}_{O2max}$ but strongly influenced adult $\dot{V}_{O2max}$.

These findings are consistent with the clinical symptoms of *A. galli* infection as described in the animal husbandry literature for domestic chickens (which were derived from ancestral Red Junglefowl). *A. galli* causes significant mortality and retards weight gain in galliform chicks, but it is not infectious and generally has very few overt effects on non-ornamental characters in adult birds (Ruff 1978; Zuk *et al.* 1990a,b). Since the mature worms live in the gut lumen, they seem unlikely to affect aerobic metabolism directly (unlike many blood-borne parasites that can reduce O_2 transport through effects on erythrocytes, such as malaria; e.g. Schall *et al.* 1982).

The difference between male and female $\dot{V}_{O2max}$ (which is also present in domestic fowl; Brackenbury

exercise instead of flight (because the muscles powering flight are different and often larger than the muscles powering running). We judged the efficacy of the running wheel protocol from several lines of evidence. First, the mean $\dot{V}_{O2max}$ we measured in Red Junglefowl was 9·6-fold larger than minimum metabolism in males and 5·6-fold higher than minimum metabolism in females. Old males can attain a mass of 2·0 kg, and the predicted ratio of $\dot{V}_{O2max}/\dot{V}_{O2mr}$ for a bird this large is 11·7. These ratios (factorial aerobic scope) are well within the range of aerobic scopes of birds in flapping flight (Rayner 1982; Hinds *et al.* 1993), and the scope for adult males is substantially greater than the maximum reported avian thermogenic scope (Marsh & Dawson 1989). Second, the aerobic scope of male Red Junglefowl is considerably greater than reported for most running birds (~3–5) and is quite similar to the highest scopes reported for trained and exercise-conditioned birds undergoing

Table 2. Repeatability (r) of $\dot{V}_{O2max}$ across different time intervals in adult Red Junglefowl, *Gallus gallus*. Repeatability was assessed from correlations of initial and final residuals generated from regressions of $\log_{10}$ mass and $\log_{10} \dot{V}_{O2max}$. Separate regressions were used to compute residuals for males and females (Table 1); the residuals from both sexes were combined for the analyses shown here. No females were used for the 2-h tests; eight females were used in each of the longer-term tests. For all time intervals, repeatability (the correlation coefficient r) was strongly positive for both sexes. A sequential Bonferroni test was applied to correct for type I errors; repeatability was significant over all four time intervals

Period	n	F	r	P
2 hours	11	38·4	0·900	0·000159
28 ± 1 days	31	125	0·901	<0·00001
56 ± 2 days	30	181	0·930	<0·00001
180 ± 7 days	22	7·29	0·517	0·0137

Table 3. Repeatability (r) of $\dot{V}_{O2max}$ of Red Junglefowl *(Gallus gallus)* across growth from age 4 weeks to reproductive maturity (age 9 months). Repeatability was assessed by regressing initial and final residuals generated from regressions of $\log_{10}$mass and $\log_{10}$ $\dot{V}_{O2max}$ for each age group (separate regressions were used for male and female adults; Table 1). A subset of chicks was inoculated with the intestinal nematode parasite *Ascaridia galli*. There was no significant repeatability of $\dot{V}_{O2max}$ in any treatment group, or in the combined data

Sex	Parasites	n	F	r	P
Male	No	13	0·25	0·149	0·63
	Yes	20	3·21	0·389	0·09
Female	No	5	1·14	0·524	0·36
	Yes	14	0·07	0·080	0·79
Combined	No	18	0·13	0·091	0·72
	Yes	34	1·98	0·241	0·17
	All	52	1·89	−0·191	0·18

& El-Sayed 1985), is impressively large and begs an explanation. Red Junglefowl are ground-dwelling forest birds from warm tropical habitats and have little need for intense thermogenic $\dot{V}_{O2}$. They do not fly for long periods and probably rely primarily on anaerobic pathways for strenuous physical activities such as escape from predators. However, males compete aggressively among themselves for territory, social status and access to females. Combatants routinely fight to exhaustion (Ligon *et al.* 1990; Johnsen & Zuk 1995), a behaviour exploited by humans in staged 'cockfights'. During prolonged contests, male junglefowl with high $\dot{V}_{O2max}$ are likely to have an advantage over opponents with lower aerobic capacity. The benefit of high $\dot{V}_{O2max}$ during sustained exercise is illustrated by the finding that male domestic fowl (whose aerobic scope is about twofold greater than that of females) have maximal sustainable running speeds of about 9 km h^{-1}, compared with about 5 km h^{-1} for females (Brackenbury & El-Sayed 1985). Accordingly, we speculate that high metabolic demand during intermale combat, coupled with low requirements for aerobic performance in other contexts, may explain the much greater $\dot{V}_{O2max}$ of adult males compared with that of adult females (although females fight among themselves, they do so with much less intensity than occurs in intermale combat). It is interesting that males and females have similar $\dot{V}_{O2mr}$ but very different $\dot{V}_{O2max}$, since one major theory of the evolution of endothermy (the aerobic capacity model; Bennett & Ruben 1979) assumes a tight coupling between minimal and maximal aerobic metabolism.

The scaling of maximum aerobic capacity during maturation in red junglefowl is greater than predicted by most allometric models. Mass exponents for both males (1·108) and females (0·919) were significantly higher than reported values for avian $\dot{V}_{O2mr}$ (~0·67 to 0·75; Lasiewski & Dawson 1967; Aschoff & Pohl

1970; Heusner 1985; Bucher 1987). Measured $\dot{V}_{O2max}$ at mean adult mass exceeded predictions extrapolated from regression of $\dot{V}_{O2max}$ on mass for uninfected chicks (Fig. 1; Table 1) by 2·6-fold in males and 1·6-fold in females. The high mass exponents for $\dot{V}_{O2max}$ may be a result of genetically induced physiological changes during maturation (probably including increased androgen synthesis in males), exercise conditioning during the period of growth, or a combination of the two. In adult mammals, $\dot{V}_{O2max}$ is generally a constant multiple of $\dot{V}_{O2mr}$ (Taylor *et al.* 1981; Hinds *et al.* 1993), but at least one study demonstrated increasing aerobic scope during post-weaning maturation (Chappell & Bachman 1995).

VARIANCE AND REPEATABILITY OF AEROBIC PERFORMANCE

The variance we observed in Red Junglefowl $\dot{V}_{O2}$ (10–15%) is approximately the same as reported for aerobic metabolism in Belding's Ground Squirrels (Chappell & Bachman 1995) and for sprint speed in several lizard and mammal species (Huey & Dunham 1987; Djawdan & Garland 1988; Van Berkum *et al.* 1989). Variance in junglefowl aerobic performance is substantially less than variance in the sprint speed and endurance of salamanders (Austin & Shaffer 1992). Nevertheless, the performance variation in junglefowl, if repeatable and heritable, would likely permit evolutionary change if subjected to directional natural selection.

It is difficult to place the repeatability of aerobic performance in Red Junglefowl into a comparative context because to our knowledge $\dot{V}_{O2max}$ repeatability has not been previously described for any bird, and data are available for only three vertebrate species. Over short periods (one day), $\dot{V}_{O2max}$ is highly repeatable in young Garter Snakes (Garland & Bennett 1990). In captive Deer Mice, $\dot{V}_{O2max}$ is repeatable across intervals ranging from 1 day to several months, and after acclimation to cold and to altitude changes (Hayes 1989a; Hayes & Chappell 1990). Free-living Belding's Ground Squirrels show significant repeatability of $\dot{V}_{O2max}$ over periods from 2 h to as long as 1–2 years, but results from a small data set suggest that there is no repeatability between the juvenile and adult life stages (Chappell *et al.* 1995). In general, our results for Red Junglefowl closely resemble the data for Belding's Ground Squirrels, although repeatabilities for adult junglefowl were usually higher than those for squirrels for sample intervals greater than 2 h.

In a broader context, the repeatability of $\dot{V}_{O2max}$ in adult junglefowl is qualitatively similar to that of locomotor performance in reptiles, amphibians and mammals: high repeatability over relatively short intervals (hours or days), with declining repeatability over longer periods (Tolley *et al.* 1983; Huey & Dunham 1987; Van Berkum et al. 1989). In contrast to

junglefowl $\dot{V}_{O2max}$, sprint speed is significantly repeatable across ontogeny in some lizards (Huey & Dunham 1987; Van Berkum et al. 1989). However, there is little or long-term repeatability of sprint speed in Garter Snakes *(T. sirtalis,* Jayne & Bennett 1990) and Tiger Salamanders *(Ambystoma californiense,* Austin & Shaffer 1992).

Although there may be costs associated with a large aerobic capacity, the adaptive value of high metabolic performance seems intuitively obvious for many animals. We have suggested that high aerobic performance is valuable for male Red Junglefowl during intermale combat (although the hypothesis has not been experimentally validated). However, even strong selection will not produce evolutionary change unless the trait in question has variance that is repeatable and heritable (repeatability sets an upper limit to heritability). Aerobic metabolism shows substantial variance in Red Junglefowl, and the repeatability of $\dot{V}_{O2max}$ in adults is among the highest yet reported for any physiological trait. Thus $\dot{V}_{O2max}$ may contain additive genetic variance, and at least partially satisfies the conditions for evolutionary change under natural selection. However, the lack of repeatability after growth from chicks to adults is problematic, and begs the question of whether adult $\dot{V}_{O2max}$ is heritable. It is conceivable that aerobic performance is highly plastic in young birds but developmentally converges upon a heritable value in older chicks and adults, even though there is no repeatability across the transition from young chicks to adults. Quantitative genetic studies could resolve this question. In any event, it is not valid to use the performance of a young Red Junglefowl to predict its aerobic capabilities after maturation.

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