THE RELATIONSHIP BETWEEN BEHAVIOUR AND MORPHOMETRIC TRAITS IN YOUNG CHICKENS

A. A. TOYE[*,1,2] AND T.B. RAJI[1]

[1]*Quantitative, Molecular and Functional Genetics Group, Department of Animal Production, Faculty of Agriculture, University of Ilorin, Kwara State, Nigeria.*

[2]*Institute for Molecular Science and Biotechnology, University of Ilorin, Kwara State, Nigeria.*

* *Correspondence: ayo.toye@btinternet.com; ayo.a.toye@gmail.com, +234 8131011392*

ABSTRACT

The correlation between behavioural (anxiety, sociality and cognition in Open Field, T-Maze, Forced Approach and Voluntary Approach tests) traits on the one hand and morphometric traits including body weight, length of body parts (Body length, Body girth, Shank length, Keel length, Wing length) and associated growth rates, Body mass Index, and Body girth: Body length ratio) from age 1 to age 8 weeks on the other, was examined in a cohort of 44 chickens consisting of 22 local (Yoruba Nigerian Local Chicken) and 22 exotic (11 Hubbard Broiler and 11 Isa Brown Layer) chickens. The levels of correlation between 160 directly measured and derived traits (of which 44 were behavioural) was determined by the use of the Pearson's method. A matrix of correlation coefficients of all 12,720 possible unique pairwise comparisons was generated. Of the 5104 Behavioural *vs.* Morphometric trait correlations, Low ($-0.3 < r < 0.3$), Medium ($0.3 < r < 0.7$ and $-0.3 > r < -0.7$) and High ($0.7 < r < 1.0$ and $-0.7 > r < -0.7$) inter-trait correlations were observed in 83%, 16% and 1% of cases respectively, and the highest Positive and Negative Coefficients observed were – 0.75 and + 0.75 respectively By contrast, in the 6730 unique Behavioural *vs.* Behavioural plus Morphometric *vs.* Morphometric trait correlations, Low, Moderate and High inter-trait correlations were observed in 18%, 26% and 56% of cases respectively, and the highest Positive and Negative Coefficients observed were – 1.00 and + 1.00 respectively. Consideration of absolute coefficient values revealed a median coefficient of 0.27 (Low Correlation). All coefficients of correlation equal to or exceeding +0.33 / - 0.33 were significant ($p < 0.05$). The highest positive coefficients of correlations ($r \geq 0.7$, High Positive Correlation) between behavioural and non-behavioural traits correspond to a link between Latency to cross the first square in an Open Field Test (which embodies anxiety and locomotory components) at age 7 weeks and Body size (BW, BL, SL, WL, BG) as shown in Table 2 and Figure 1. High coefficients of correlation were also observed between Latency to cross the first square in an Open Field Test (which embodies anxiety and locomotory components) at age 7 days and Body size (BW, WL, KL, SL, BMI) as shown in Table 2 and Figure 1. In addition, high positive correlation ($r \geq 0.7$) was observed between Time resting in an OFT at age 48 days on the one hand, and body size (BW, BG, GLR, BMI) as shown in Table 2 and Figure 1. The high negative coefficients of correlation occurred between Time ambulating in an Open Field Test (Behavioural traits) at age 6 weeks on the one hand and body weight, body shape and fatness indices (Morphometric traits) including BW, BG. GLR and BMI (Figure 1 and Table 2). These data point to the potential utility of morphometric traits as heurictic/surrogate markers for behavioural traits in poultry breeding and management. Further, they suggest that determinants of activity in a stressful environment may determine body weight and size of chickens as has been observed in humans and laboratory animals with consequences for the incidence and progression of non-communicable metabolic dysfunction/diseases and their sequelae., and/or alternatively, that determinants of early body weight and size determine activity in under anxiety inducing stress, as has been shown in the relationship between obesity and stress related health conditions in humans. Further studies will partition the genotype and environmental influences on the high correlation between behaviour and morphometric traits reported here, and will define the underlying molecular basis of the former.

Keywords: Behavioural traits, morphometric traits, correlation, concordance, discordance, chicken.

INTRODUCTION

Recent ethological studies have shown that local and exotic chickens differ in behaviour (anxiety, sociality and cognition). Independent studies have shown that local and exotic chickens differ in body weight and size as measured by the length of various body parts, and associated growth rates. The link between body weight and length of body parts on the one hand, and behaviour on the other has received less attention in chickens. Evidence from other species (humans and model laboratory animals including rats and mice) has however demonstrated links between behaviour and body size, particularly in the context of health. In this context, several studies have shown that sedentary behaviour coupled with feeding behaviour which promotes excessive intake of energy rich foods leads to an energy input: energy expenditure imbalance in favour of the former, ultimately promoting obesity. In turn, obesity is associated with/linked to a wide constellation of non-communicable metabolic diseases (Metabolic syndrome, Type 2 diabetes, fatty liver disease, coronary artery/cardiovascular disease, cancer etc), chiefly through its effect in promoting insulin insufficiency, low grade inflammation, oxidative stress and hyper-cholesterolaemia. Whereas these studies on metabolic disease have pointed an association of behaviour with metabolic disease risk in the context of its effect on energy balance, independent studies in the fields of behaviour and mental health have revealed that diseases/problems of energy balance such as obesity are associated with anxiety and other adverse states of mental health.

Knowledge of correlation between behaviour and mass/size-related (morphometric) traits can lead to the discovery of markers/ predictors of behaviour and enhance understanding of the likely response of behavioural traits to primary selection for morphometric traits.

The current study examines the relationships between behaviour (anxiety, sociality and cognition sub-phenotypes) and physical form (Morphometric measurements) through studies of their correlation.

MATERIALS AND METHODS

Animals: A cohort of 44 birds consisting of 22 local birds (Yoruba Nigerian Local Chicken) and 22 exotic birds (11 Hubbard Broiler and 11 Isa Brown Layer chickens) were studied. All birds were housed in conventional cages, granted unrestricted access to drinking water and fed *ad libitum* (Broiler feed from Top Feeds, Ilorin .Nigeria) from age 1 – 8 weeks.

Mass/size-related (morphometric) traits: Body weights and lengths of body parts were determined weekly from age 1 – 8 weeks as described previously (Toye *et al.*, 2013) and summarised below.

Body weight (BW): Body weight in gram (g) was recorded to two decimal places, by use of a sensitive weighing scale (Scout II brand)

Body length (BL): Body length, was taken as the nostril to pygostyle distance measured in centimetre (cm) units when a tape measure is stretched from a bird's nasal opening, along its gently stretched neck, and along its back, to the tip of its pygostyle.

Body girth (BG): Body girth, was taken as the distance in centimetre (cm) units covered when a tape measure is looped round the region of the breast, taking care to run the tape under (rather than over) the Wing.

Shank length (SL): The shank length was taken as the distance in centimetres (cm) between the foot pad and the hock joint, measured by use of a set of Venire callipers.

Keel length (KL): This was taken as the distance between the cranial and caudal termini of the Keel bone, measured in centimetre (cm) units by use of a tape measure.

Wing length (WL): Wing length was taken as the distance from the humerus-coracoid junction to the distal tip of the phalange digits, and was measured in centimetre (cm) units by the use of a tape measure.

Body Girth: Body Length Ratio (GLR): This was taken as the ratio of the body girth in centimetre units to body length in centimetre and determined weekly from age 1 – 7 weeks.

GLR = BG (cm) / BL (cm) .... (1)

Body Mass Index (BMI): This was taken as the ratio of the body weight in gram units to the square of the body length in centimetre units, and determined weekly from age 1 – 7 weeks.

BMI = BW (g) / BL^2 .... (2)

Growth rate: For body weight (g), and each of the linear measures of body growth recorded (cm), growth rate/velocity/potential for 7 day intervals (week 1 - 2, 2 - 3, 3 - 4, 4 - 5, 5 – 6, and 6 – 7), 21 day intervals (week 1 – 4 and Week 4 - 7), and a 42 day interval (Week 1 – 7) were determined by the use of an adapted version of the formula of Maciejowski and Ziêba (1982),

$I = [(T2 - T1) / (T2 + T1)/2] \times 100$ .... (3)

Where: I= Growth rate per unit period (%). T1= Average initial reading for the stated period (g or cm). T2= Average final reading for the stated period (g or cm).

Behavioural Traits Locomotory activity in the context of exposure to an anxiogenic environment is a reporter of (sub-phenotype of) anxiety, and a pro-survival behaviour. Vocalisation in the context of exposure to an anxiogenic environment is a reporter of (sub-phenotype of) anxiety, and a pro-social and pro-survival behaviour. Also, the time taken to restore visual contact and proximity with con-specifics in the context of exposure to an anxiogenic environment is a reporter of (sub-phenotype of) anxiety, and a pro-social and pro-survival behaviour.

Each bird was subjected to a battery of ethological tests of fearfulness, anxiety and cognition, including the Open Field Test (OFT) at age 7 days and a repeat test at 48 days, T-Maze test at 2 weeks age and a repeat test at 7 weeks age, a Voluntary Approach Test at age 4 weeks and a repeat test at age 8 weeks, and a Forced Approach test at age 4 weeks and a repeat test at age 8 weeks as described previously (Raji and Toye, 2014).

Forty four (44) traits were examined, including:

- Latency to cross the first square in an OFT apparatus during Test 1 at age 7 days and Test 2 at age 48 days (Latency_OFT1 and Latency_OFT2 respectively).
- Total time spent walking in an OFT apparatus during Test 1 at age 7 days and Test 2 at age 48 days (Total_Time_OFT1_Ambul and Total_Time_OFT2_Ambul respectively).
- Total time spent resting in an OFT apparatus during Test 1 at age 7 days and Test 2 at age 48 days (Total_Time_OFT1_Rest and Total_Time_OFT2_Rest respectively).
- Total number of squares crossed in an OFT apparatus during Test 1 at age 7 days and Test 2 at age 48 days (No_of_Squares_OFT1 and No_of_Squares_OFT2 respectively).
- Total number of distress calls in an OFT apparatus during Test 1 at age 7 days and Test 2 at age 48 days (No_of_Distress_Calls_OFT1 and No_of_Distress_Calls_OFT2 respectively).
- Latency (period spent) in the starting square of a T-Maze Apparatus in Sessions 1 – 5 of Test 1 at age 2 weeks and Test 2 at age 7 weeks (Latency_T1_S1; Latency_T1_S2; Latency_T1_S3; Latency_T1_S4; Latency_T1_S5; Latency_T2_S1; Latency_T2_S2; Latency_T2_S3; Latency_T2_S4, and; Latency_T2_S5 respectively).
- Total time spent with con-specifics in a T-Maze Apparatus in Sessions 1 – 5 of Test 1 at age 2 weeks and Test 2 at age 7 weeks (Time_Spent_T1_S1; Time_Spent_T1_S2; Time_Spent_T1_S3; Time_Spent_T1_S4; Time_Spent_T1_S5; Time_Spent_T2_S1; Time_Spent_T2_S2; Time_Spent_T2_S3; Time_Spent_T2_S4, and; Time_Spent_T2_S5 respectively).
- Time needed to find con-specifics in a T-Maze Apparatus in Sessions 1 – 5 of Test 1 at age 2 weeks and Test 2 at age 7 weeks (Time_Needed_T1_S1; Time_Needed_T1_S2; Time_Needed_T1_S3; Time_Needed_T1_S4; Time_Needed_T1_S5; Time_Needed_T2_S1; Time_Needed_T2_S2; Time_Needed_T2_S3; Time_Needed_T2_S4, and; Time_Needed_T2_S5 respectively).
- Latency to peck the food in the experimenter's hand in the Voluntary Approach Test 1 at age 4 weeks and Voluntary Approach Test 2 at age 8 weeks (VAT_T1 and VAT_T2 respectively).
- Latency to flee from the approaching experimenter in the Forced Approach Test 1 at age 4 weeks and Forced Approach Test 2 at age 8 weeks (FAT_T1 and FAT_T2 respectively).

Statistical Analysis

Pearson's bivariate correlation: The levels of correlation between behavioural and non-behavioural traits were determined by the use of the Pearson's method (Falconer, 1989) implemented in SPSS 21 (IBM SPSS, 2008):

$r = Cov(X,Y) / \sqrt{(Var(X) \cdot Var(Y))}$ ....(4).

Where: r = coefficient of correlation. X = Phenotypic Trait 1. Y = Phenotypic trait 2. *Cov* = Covariance. *Var* = Variance.

Absolute Correlation values between 0 and 0.3 (including the range covering 0 and -0.3), 0.3 and 0.7 (including the range covering -0.3 and -0.7) and 0.7 and 1.0 (including the range covering -0.7 and -1.0) were classed as "Low", "Medium" and "High" Correlations respectively.

RESULTS AND DISCUSSION

A total of 160 directly measured and derived traits were examined, of which 44 were behavioural traits derived from ethological tests and the remaining 116 traits were Mass/size-related (morphometric) traits measured from age 1 to age 8 weeks and derivatives thereof. In a 44 x 116 matrix corresponding to 5104 Behavioural *vs.* Mass/size-related trait correlations, termed "Self *vs.* Non-Self" correlations (extracted from a full 160 x 160 matrix of all 12,720 possible unique pairwise comparisons of the examined traits), Low, Moderate and High inter-trait correlations were observed in 83%, 16% and 1% of cases respectively. The highest Positive and Negative Coefficients observed were – 0.75 and + 0.75 respectively. Consideration of absolute coefficient values (irrespective of direction of correlation) revealed a median coefficient of 0.12 (Low Correlation). By contrast, in the 6730 unique Behavioural *vs.* Behavioural plus Morphometric *vs.* Morphometric trait correlations (termed "Self *vs.* Self" correlations), Low, Moderate and High inter-trait correlations were observed in 18%, 26% and 56% of cases respectively. The highest Positive and Negative Coefficients observed were – 1.00 and + 1.00 respectively. Consideration of absolute coefficient values revealed a median coefficient of 0.27 (Low Correlation). All coefficients of correlation equal to or exceeding +0.33 / - 0.33 were significant ($p < 0.05$).

Positive Correlation

With the exception of Squares crossed during an OFT, every one of the 10 classes of behavioural traits showed Moderate (Medium) or greater positive correlation to at least one of the 116 body weight, lengths of body parts and associated growth rate, GLR and BMI traits examined (Table 1, Figure 1). Only Latency in the T-Maze Test, Latency in the OFT, and amount of time spent resting in the OFT behavioural trait classes were highly positively correlated with one or more body weight, lengths of body parts and associated GLR and BMI traits (Table 1, Figure 1). The Maximum positive correlation of Number of Distress calls in an OFT, The time required to elicit a flight response to the approaching experimenter in a FAT, Time spent ambulating in an OFT, Time Needed to reinstate proximity and visual contact with a con-specific in a T-Maze test, Time Spent in proximity and visual contact with a con-specific in a T-Maze test, and, The time required to elicit a flight response to the approaching experimenter in a VAT was in the medium/Moderate range ($r=0.3 - 0.7$) as shown in Table 1 and Figure 1.

The Maximum positive correlation of the behavioural trait class indicating the Number of squares crossed in an OFT (the extent of exploration of the anxiogenic arena) with one or more body weight, lengths of body parts and associated growth rate, GLR and BMI traits was in the Low range ($r<0.3$) as shown in Table 1 and Figure 1.

Further examination of the data showed that Highest positive coefficients of correlations ($r \geq 0.7$, High Positive Correlation) between behavioural and non-behavioural traits correspond to a link between Latency to cross the first square in an Open Field Test (which embodies anxiety and locomotory components) at age 7 weeks and Body size (BW, BL, SL, WL, BG) as shown in Table 2 and Figure 1. High coefficients of correlation were also observed between Latency to cross the first square in an Open Field Test (which embodies anxiety and locomotory components) at age 7 days and Body size (BW, WL, KL, SL, BMI) as shown in Table 2 and Figure 1. In addition, high positive correlation ($r \geq 0.7$) was observed between Time resting in an OFT at age 48 days on the one hand, and body size (BW, BG, GLR, BMI) as shown in Table 2 and Figure 1.

Negative Correlation

Every one of the 10 behavioural trait classes showed low or greater negative correlation to at least one of the 116 body weight, lengths of body parts and associated growth rate, GLR and BMI traits examined (Table 1, Figure 1).

Of the 10 behavioural trait classes examined, only the time spent ambulating in an OFT showed high negative correlation ($r > -0.7$) with one or more of the 116 morphometric traits (body weight, length of body parts, growth rate and associated GLR and BMI) examined as shown in Table 1 and Figure 1. Each of the 9 other behavioural trait classes exhibited Moderate (medium) level correlation to one or more of the 116 Morphometric traits examined.

Further examination of the data showed that High negative coefficients of correlation occured between Time ambulating in an Open Field Test (Behavioural traits) at age 6 weeks on the one hand and body weight, body shape and fatness indices (Morphometric traits) including BW, BG, GLR and BMI (Figure 1 and Table 2).

The results showed that maxima for coefficients of correlation between related traits (Behavioural *vs.* Behavioural plus Morphometric *vs.* Morphometric correlations, termed "Self *vs.* Self" correlations) were higher than those observed for correlation between unrelated traits

(Behavioural *vs.* Morphometric trait correlations, termed "Self *vs.* Non-Self" correlations). Likewise, the proportion of coefficients within the High and Medium Correlation brackets were higher in the former than the latter group.

The morphometric traits that were highly positively correlated to behavioural traits in the present study point to a direct relationship between early growth (age 1 – 4 weeks) and anxiety as measured by latency in anxiogenic environments presented by the OFT and T-Maze ethological test environments. The recapitulation of the High correlation between latency and growth sub-phenotypes in older birds (age 4 – 8) point to a consistent pattern of relationship over the 1st 8 weeks of life examined in the present study. Equally, the morphometric traits that were highly negatively correlated to behavioural traits in the present study point to an inverse relationship between early growth and activity as measured by time ambulating in an OFT. Though the novel nature of this study precluded direct contextuation against the published research on avian biology, numerous related studies on humans support the observations reported here (Moreira *et al.*, 2007; Guedes *et al.* 2013; Esposito *et al.*, 2014; Haghighi *et al.*, 2016). Recent studies have shown that mood disorders are associated with increasing waist circumference in human (Moreira *et al.*, 2007), and that the relationship between anxiety and size/adiposity (BMI) is complex, and in humans, follows an inverted "U" shape, being lowest at either extremes of the BMI distribution (Low and Extremely High BMI groups), and higher for Medium and High BMI sub groups (Haghighi *et al.*, 2016). Further, Guedes *et al.* (2013) showed significant medium positive correlation between percentage of total body fat (Dual Energy X-ray Absorptiometry) and Anxiety in overweight and obese humans with metabolic syndrome. Esposito *et al.* (2014) also reported Medium positive correlation ($r = 0.59$) between Body Mass Index (BMI) and anxiety (Self-Administered Psychiatric Scales for Children and Adolescents, SAFA) in a case-control study of obese and normal children.

The similarity of the relationship between anxiety and body size in humans and chickens may suggest shared underlying mechanisms, and suggests that knowledge may be shared across species in efforts to unravel the genetic architecture of the link between body size and behaviour.

CONCLUSION AND RECOMMENDATIONS

These data point to the potential utility of morphometric traits as heurictic/surrogate markers for behavioural traits in poultry breeding and management. Further, they suggest that determinants of activity in a stressful environment may determine body weight and size of chickens as has been observed in humans and laboratory animals with consequences for the incidence and progression of non-communicable metabolic dysfunction/diseases and their sequelae., and/or alternatively, that determinants of early body weight and size determine activity in under anxiety inducing stress, as has been shown in the relationship between obesity and stress related health conditions in humans. The absence of high correlation between Markers of Pro-Social Behaviour/Cognition (time needed to find con-specifics, time spent with con-specifics and vocalisation/number of calls) and any of morphometric traits examined here suggests a greater complexity of determination of the latter beyond the scope of the former examined here. Further, the data indicate that the determinants of pro-survival social reinstatement/pro-social behaviour in an anxiogenic context do not completely overlap (are not unitary) with determinants of Latency and locomotory components of behaviour in the same anxiogenic contexts, as the latter but not the former were highly correlated with morphometric traits. Further studies will partition the genotype and environmental influences on the high correlation between behaviour and morphometric traits reported here, and will define the underlying molecular basis of the former.

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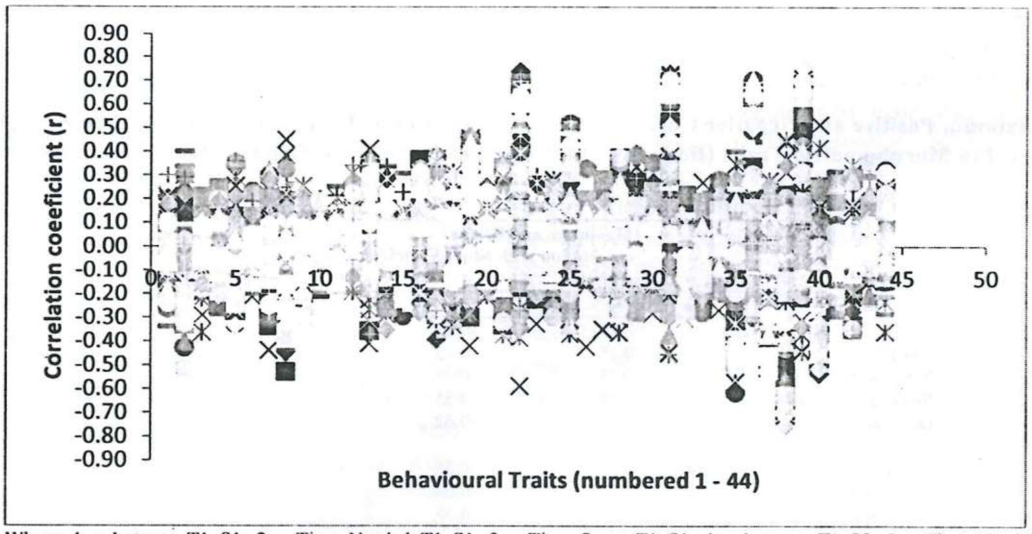
Table 1. Maximum Positive and Negative Correlation between Classes of 4 Ethological Tests (representing 44 traits) and 116 Morphometric Traits (Body Weight, Length of Body Parts, Growth Rate and associated traits).

Behaviour		Maximum coefficient (r) of correlation with Morphometric traits	
Test	Class	+ve	-ve
OFT	Latency	0.73	-0.46
	Time_Ambul	0.45	-0.75
	Time_Rest	0.75	-0.45
	Squares_Crossed	0.28	-0.55
	Distress_Calls	0.51	-0.62
T-Maze	Latency	0.74	-0.59
	Time_Needed	0.45	-0.53
	Time_Spent	0.45	-0.38
VAT	Latency	0.34	-0.38
FAT	Latency	0.36	-0.36

Table 2. Pairs of traits exhibiting high coefficients of correlation in the Behavioural vs. Morphometric trait Pearson's bivariate correlation analysis.

"r" coefficient (level and direction of relationship)	Trait 1 (Behavioural) vs.	Trait 2 (Morphometric)
High Negative Correlation (> - 0.07)	Time Ambulating in an Open field test (OFT) at age 6 weeks	BG2 (-0.7); BMI3 (-0.7); BW3 (-0.7); BMI5 (-0.7); BW5 (-0.71); GLR5 (-0.7); BW6 (-0.71); GLR6 (-0.7); BG7 (-0.71); BW7 (-0.71); GLR7 (-0.75); BG8 (-0.72); BW8 (-0.71).
High Positive Correlation (> + 0.07)	Time Resting in an Open field test (OFT) at age 48 days.	BG2 (0.7); BMI3 (0.7); BW3 (0.7); BW5 (0.71); BW6 (0.71); GLR6 (0.7); BG7 (0.71); BW7 (0.71); GLR7 (0.75); BG8 (0.72); BW8 (0.71).
	Latency to traverse the first square in an Open Field Test (OFT) at age 48 days.	BMI2 (0.71); KL4 (0.7).
	Latency to traverse the first square in an Open field test (OFT) at age 7 days.	BMI3 (0.71); BW3 (0.71); BMI5 (0.7); BW5 (0.73); WL5 (0.72); BMI6 (0.72); BW6 (0.73); KL6 (0.7); SL6 (0.71); WL6 (0.71); BMI7 (0.7); BW7 (0.73); WL7 (0.72); BW8 (0.73).
	Latency to traverse the first square in Session 3 of the second T-Maze test at age 7 weeks.	BW1 (0.73); BL2 (0.7); BW2 (0.7); SL2 (0.7); WL2 (0.74); WL4 (0.7); BW6 (0.7); BW7 (0.71); WL7 (0.71); BG8 (0.72); BW8 (0.72);

BW = Body weight (g); BL = Body length (cm); BG = Body Girth (cm); WL = Wing Length (cm); SL = Shank Length (cm); KL = Keel Length (cm); BMI = Body Mass Index; GLR = Body Girth: Body Weight ratio; 1,2,3…8 = age in weeks at which trait was measured; (x) = Numbers in parentheses are coefficients of correlation, ranging from -1 to +1.



Where: 1 = Latency_T1_S1; 2 = Time_Needed_T1_S1; 3 = Time_Spent_T1_S1; 4 = Latency_T1_S2; 5 = Time_Needed_T1_S2; 6 = Time_Spent_T1_S2; 7 = Latency_T1_S3; 8 = Time_Needed_T1_S3; 9 = Time_Spent_T1_S3; 10 = Latency_T1_S4; 11 = Time_Needed_T1_S4; 12 = Time_Spent_T1_S4; 13 = Latency_T1_S5; 14 = Time_Needed_T1_S5; 15 = Time_Spent_T1_S5; 16 = Latency_T2_S1; 17 = Time_Needed_T2_S1; 18 = Time_Spent_T2_S1; 19 = Latency_T2_S2; 20 = Time_Needed_T2_S2; 21 = Time_Spent_T2_S2; 22 = Latency_T2_S3; 23 = Time_Needed_T2_S3; 24 = Time_Spent_T2_S3; 25 = Latency_T2_S4; 26 = Time_Needed_T2_S4; 27 = Time_Spent_T2_S4; 28 = Latency_T2_S5; 29 = Time_Needed_T2_S5; 30 = Time_Spent_T2_S5; 31 = Latency_OFT1; 32 = No_of_Squares_OFT1; 33 = Total_Time_OFT1_Ambul; 34 = Total_Time_OFT1_rest; 35 = No_of_Distress_Calls_OFT1; 36 = Latency_OFT2; 37 = No_of_Squares_OFT2; 38 = Total_Time_OFT2_Ambul; 39 = Total_Time_OFT2_rest; 40 = No_of_Distress_Calls_OFT2; 41 = VAT_T1; 42 = VAT_T2; 43 = FAT_T1; 44 = FAT_T2.

Figure 1. Coefficients of Relationship between Trait pairs examined, and coefficient of correlation in the Behavioural vs. Morphometric trait Pearson's bivariate correlation analysis.