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Evolutionary design of a flexible, seasonally migratory, avian phenotype: why trade gizzard mass against pectoral muscle mass?

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Migratory birds undergo impressive body remodelling over the course of an annual cycle. Prior to long-distance flights, red knots (*Calidris canutus islandica*) reduce gizzard mass while increasing body mass and pectoral muscle mass. Although body mass and pectoral muscle mass are functionally linked via their joint effects on flight performance, gizzard and pectoral muscle mass are thought to be independently regulated. Current hypotheses for observed negative within-individual covariation between gizzard and pectoral muscle mass in free-living knots are based on a common factor (e.g. migration) simultaneously affecting both traits, and/or protein limitation forcing allocation decisions. We used diet manipulations to generate within-individual variation in gizzard mass and test for independence between gizzard and pectoral muscle mass within individuals outside the period of migration and under conditions of high protein availability. Contrary to our prediction, we observed a negative within-individual covariation between gizzard and pectoral muscle mass. We discuss this result as a potential outcome of an evolved mechanism underlying body remodelling associated with migration. Although our proposed mechanism requires empirical testing, this study echoes earlier calls for greater integration of studies of function and mechanism, and in particular, the need for more explicit consideration of the evolution of mechanisms underlying phenotypic design.

1. Introduction

Understanding the causes and consequences of phenotypic variation is a central aim in ecology and evolution [1,2]. Organisms are made up of multiple traits which show varying degrees of inter-dependence. To account for this complexity, it is becoming increasingly common for researchers to adopt a multi-trait approach to understanding phenotypic variation [3–7]. Positive covariances between traits are suggestive of functional integration (i.e. phenotypic integration) [8,9], while negative covariances are suggestive of trade-offs [10]. For example, flight performance in birds is determined by overall body mass and the size of the pectoral muscle [11,12]. All else being equal, birds that are heavier require larger pectoral muscles to achieve the same flight performance as lighter birds [11–13]. This functional association between body mass and pectoral muscle mass manifests as a positive covariance between the two traits (e.g. [14–16]). Traits can also be linked via trade-offs when organisms have

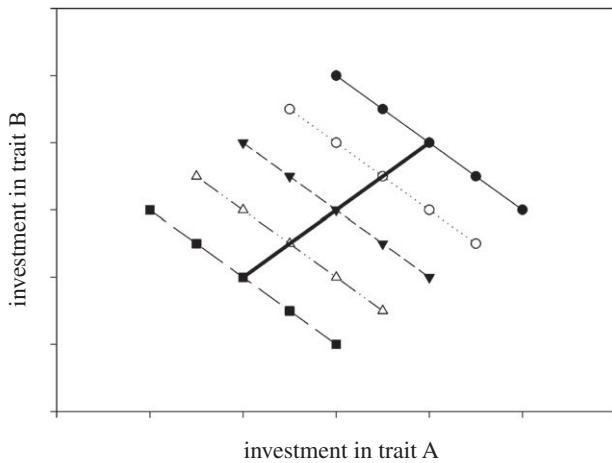


Figure 1. Illustration of trait covariances that differ at different levels of organization. Individuals are represented by different symbols and each individual is represented by five points and a thin line. At the within-individual level, a higher investment in trait A is associated with a lower investment in trait B. However, across individuals, the relationship is reversed. Individuals that have a higher average expression of trait A also have a higher average expression of trait B. The thick black line shows the relationship across the average values for each individual.

limited resources that must be allocated to competing processes [10,17–19]. For example, under conditions with limited access to dietary protein, higher protein investment towards one muscle group must come at the cost of investment in another muscle group [20–22].

For labile phenotypic traits, patterns of covariation between traits can vary across different levels of organization (e.g. within versus among individuals) [23]. For example, when resources (e.g. time or energy) are limited, the more an individual invests in trait A, the less resource is available to invest in trait B. This generates negative covariation between traits A and B at the within-individual level. However, individuals may differ in how much resource they have available to them, for example, because some individuals are on higher quality territories than others. Individuals with access to more resource can invest more on average in both trait A and trait B [24]. Individual differences in access to resource shape positive correlations between traits A and B at the among-individual level (figure 1). When the relative importance of different mechanisms in shaping patterns of covariation differs at the within- versus among-individual level, unpartitioned phenotypic correlations can obscure level-specific relationships [24–26]. Thus, studying the covariance structure of multiple traits and partitioning phenotypic covariations to the within- and the among-individual levels can provide insights regarding level-specific mechanisms shaping patterns of trait co-variation [26–29]. However, studying trait integration at the within-individual level requires study systems for which it is possible to repeatedly measure the traits of interest in the same individual [26]. Although this is relatively straightforward for behavioural traits and certain physiological traits (e.g. metabolic rates, hormone levels, etc.), studies on integration of internal structures (e.g. organ size) are rare because of the methodological limitations of repeatedly assaying such traits in the same individuals [30–32]. Red knots (*Calidris canutus*), a medium-sized migratory shorebird, provide a rare opportunity to study level-specific trait covariation of internal traits, because non-destructive methods have been developed

that allow for accurate estimation of internal organs [30]. Red knots have also served as good experimental animals to test for seasonally changing phenotypic traits that are related to migration but are maintained in captivity [33,34], together with the bodily consequences of differences in ecological contexts such as ambient temperature [35,36], predation risk [16] and diet ([37], this study).

In this study, we focus on three ecologically important traits for knots: body mass (size, storage capacity, resource holding potential [38]), pectoral muscle mass (flight performance [11,12,15,16]) and gizzard mass (digestive performance, [39,40]). At the among-individual level, we predicted positive covariation between all three traits if they are simple expressions of body size. At the within-individual level, we predicted different trait covariance structures. Body mass and pectoral muscle mass are functionally linked through their concurrent effects on flight [41], and previous studies in free-living knots have reported positive within-individual covariation between these two traits (e.g. [14]). By contrast, gizzard mass and pectoral muscle mass are probably independently regulated traits. Nevertheless, a negative within-individual covariation was reported between body mass and gizzard mass [19,33,42], and between pectoral muscle mass and gizzard mass [19,33,43] as a result of body remodelling associated with migration. These patterns are similar to patterns that have been observed in other birds [1,20,31,32,44–46]. Current hypotheses for the observed negative covariation between gizzard mass and pectoral muscle mass in free-living knots are based on a common factor (i.e. migration) simultaneously affecting both traits [19,22,31,44,47], protein limitation forcing allocation decisions between gizzard mass and pectoral muscle mass [19–21], or a combination of the two. Thus, outside the migratory season and under conditions of high protein availability, gizzard mass and pectoral muscle mass are predicted to vary independent of one another at the within-individual level.

We used diet manipulations to generate within-individual variation in gizzard mass and test for the independence of gizzard mass and pectoral muscle mass at the within-individual level. Knots are mollusc eating shorebirds that swallow their prey whole [48]. Prey are crushed in the muscular stomach (i.e. gizzard). Gizzard mass is flexible; individuals adjust their gizzard mass over the course of days in response to changes in the digestive quality (i.e. the ratio of digestible to indigestible part) of their diets such that when individuals consume a diet of high digestive quality (i.e. with a high ratio of digestible to indigestible parts) they develop smaller gizzards, and when they consume a diet of low digestive quality they develop large gizzards [49–51]. Manipulations of gizzard mass were performed over two successive non-breeding seasons, under conditions of ad libitum access to protein-rich food. We predicted that experimental manipulation of gizzard mass would not induce any systematic change in pectoral muscle mass.

2. Methods

(a) Study subjects

Red knots of the *islandica* subspecies (e.g. [52]) were captured using mist nests on the mudflats of Richel (53°16' N, 05°23' E), Griend (53°14' N, 05°15' E) and Schiermonikoog (53°28' N, 06°10' E) in the Wadden Sea, The Netherlands, between August and October

2015 and transported to the experimental shorebird facilities at the NIOZ Royal Netherlands Institute for Sea Research. Outside of the experiments (April through to October), birds were housed in eight aviaries in flocks of between 14 and 17 individuals, and were maintained on an ad libitum diet of protein-rich trout pellets (Trouvit, Produits Trouw, Vervins, France). Birds were handled each week to assess overall health [53] and to allow the aviaries to be cleaned. Further details on husbandry conditions are provided in the electronic supplementary material, text S1.

(b) Experimental procedure

Experiments were conducted between October and March in each year, which is outside the migration period of *islandica* red knots [15]. During this time, birds experienced eight diet manipulations (four in each year, see below) with concurrent measurements of body mass, gizzard mass and pectoral muscle mass. Diet manipulations were used to generate within-individual variation in gizzard mass [51]. To induce small gizzards, knots were maintained on an ad libitum diet of Trouvit, the same high digestive quality food as provided outside of the experiments. Trouvit pellets are 47% protein by dry weight. To induce large gizzards, knots were fed an ad libitum staple diet of thawed mudsnails, *Peringia ulvae* (formerly *Hydrobia ulvae*). Knots offered a staple diet of *Peringia* consume them whole, thereby ingesting a large fraction of indigestible material. Thus, *Peringia* is of low digestive quality and induces gizzard hypertrophy (e.g. [39,54,55]). Although we did not obtain measures for the protein content of the *Peringia* used in our experiments, molluscs are generally recognized as being a protein-rich food (range 50% to greater than 80% protein by dry weight) [56–58]. Furthermore, in other studies, knots have been kept on diets of *Peringia* for extended periods (three to 12 months) [16,54,59], with no evidence of loss of body condition, as would be expected if this food was low in protein. This was also true in the present study; knots were maintained on *Peringia* diets repeatedly for up to six consecutive weeks with no evidence of loss of body condition (K.J. Mathot, E.M.A. Kok 2016, personal observation).

In each year of the experiments, all individuals were subjected to two replicates of the *Peringia* diet treatment (P) and two replicates of the Trouvit diet treatment (T) in alternating sequence. Half of the aviaries (and therefore half of the birds) were randomly assigned to the PTPT treatment order, and the remaining half of aviaries to TPTP treatment order. By using a balanced crossover design, we ensured that experimentally induced variation in gizzard mass was not confounded with any circannual endogenous rhythms in any of the three focal traits [34,60].

Previous work has shown that gizzard mass adjusts to the digestive quality of the diet within 6–14 days [51]. To ensure that gizzard mass was stable over the duration of physiological measurements in a given treatment block, we allowed birds to remain on staple diets for at least three weeks before commencing physiological measurements. Gizzard mass and pectoral muscle mass were measured by A.D. using an ultrasound scanner (model Aquila, Pie Medical Benelux, Maastricht, The Netherlands). Detailed descriptions of the procedure are provided elsewhere ([30,51], electronic supplementary material, text S2). Following physiological measurements, birds were subjected to behavioural observations as part of another study [61]. These observations required an additional three weeks on the staple diet, resulting in up to six consecutive weeks on any given diet treatment.

(c) Data selection and statistical analyses

Some knots did not experience the experimentally determined diet manipulation sequence because some individuals did not adjust to switches to a *Peringia* diet (i.e. the low digestive quality diet) ($n = 3$). These individuals were excluded because their physiological trait values may have been in flux (see [62]). Similarly, birds with

Staphylococcus infections had missing observations as we did not measure trait values in birds that were being treated for active infections ($n = 5$ cases). Thus, the final sample size for the analyses presented here are: $n = 584$ observations, $n = 88$ individuals.

Our experimental subjects included birds caught as juveniles ($n = 44$) and birds captured as adults ($n = 44$) and spanned two successive non-breeding seasons. We considered the possibility that covariance structures would be year- or cohort-specific, and therefore initially ran four separate multivariate models. However, there was no support for differences between the covariance matrices (see the electronic supplementary material, table S1). Therefore, for simplicity, analyses presented in the main text are from a single multivariate model, including data from both years and both age cohorts.

We estimated the among- and within-individual correlations by fitting gizzard mass, pectoral muscle mass and body mass as three response variables using the *MCMCglmm* function [63] in the R statistical environment [64] and modelling random intercepts for bird identity. We did not include random intercept for aviary identity because univariate analyses revealed this to be of minor importance for all three traits (analyses not shown). See the electronic supplementary material, text S3 for further details on *MCMCglmm* implementation procedures.

Because total body mass includes gizzard mass and pectoral muscle mass, positive correlations may have resulted from part-whole correlations (see [65] for discussion). We addressed this possibility by repeating analyses using body mass minus pectoral muscle mass to estimate correlations between pectoral mass-independent body mass and pectoral muscle mass, or body mass minus gizzard mass to estimate correlations between gizzard mass-independent body mass and gizzard mass. We compared the results of these analyses with results from analyses using overall body mass and present both.

We predicted positive among-individual covariance between body mass, gizzard mass and pectoral muscle mass if each of these traits are expressions of overall body size. To further assess this possibility, we tested whether an individual's average gizzard mass, average body mass and average pectoral muscle mass correlated with their structural body size. For 68 individuals, we had complete data on three measures of structural body size (bill length, wing length and tarsus length), as well as sufficient measures of body mass, gizzard mass and pectoral muscle mass (minimum four per individual, two on each diet treatment) to calculate an individual's average value for each of these traits. Following earlier studies [55,66], we used the first principal component (PC1) of a principal component analysis of wing length, tarsus length and bill length as our measure of structural body size using the *prcomp* function. Next, we estimated pairwise correlations (estimate and 95% confidence interval) between average body mass, average gizzard mass and average pectoral muscle mass using the *corr.test* function. Because our study design prevented any confounding relationship between time of year (e.g. relative to migration, photoperiod, temperature, etc.) and gizzard mass, within-individual relationships between gizzard mass and pectoral muscle mass or body mass could not be owing to time of year effects, and therefore, we did not correct for time of year in these analyses.

Next, we evaluated the amount of within-individual variance for each of our three focal traits (gizzard mass, pectoral muscle mass and body mass) that could be explained by our experimental diet treatments. To do this, we constructed univariate mixed effects models with either gizzard mass, pectoral muscle mass or body mass as a response variable, and diet treatment as a fixed effect. Bird ID was included as a random effect. We then used the 'r.squaredGLMM' function from the 'MuMIn' package in R which calculates the per cent of variation in the response variable explained by fixed effects.

Finally, we estimated the slopes of the within- and among-individual correlations using two datasets: an among-individual

Table 1. Covariance matrix for body mass, pectoral muscle mass and gizzard mass in red knots. (Values above the diagonal are among-individual correlations, values below are within-individual correlations. Estimates presented in italics are from identical models run using body mass independent of the muscle type for which the correlation is estimated (e.g. for correlation between body mass and gizzard mass, gizzard mass is subtracted from total body mass).)

	body mass (g)	pectoral muscle mass (g)	gizzard mass (g)
body mass (g)	—	0.79 (0.67, 0.87) <i>0.56 (0.51, 0.62)</i>	0.85 (0.63, 0.97) <i>0.85 (0.63, 0.98)</i>
pectoral muscle mass (g)	0.54 (0.48, 0.60) <i>0.36 (0.29, 0.44)</i>	—	0.79 (0.53, 0.96)
gizzard mass (g)	0.13 (0.04, 0.21) <i>-0.03 (-0.11, 0.05)</i>	-0.13 (-0.22, 0.05)	—

dataset, and a within-individual dataset. The among-individual dataset was comprised of average trait values per individual estimated across all repeated measures. The within-individual dataset was comprised of within-individual centred data in which each individual's mean trait value was subtracted from each observation for that individual [67]. As all variables have associated measurement error, we used reduced major axis (RMA) regression to estimate slopes between pairs of traits using the *lmodel2* function from the 'lmodel2' package.

For all analyses, we evaluated support for effects based on estimated effects sizes and their 95% credible intervals (CIs) [68,69]. This approach has been advocated to avoid drawing dichotomous conclusions to accept or reject the null hypothesis based on data which can show a continuous range of support (or lack of support) for a given interpretation [69–71]. However, for readers less familiar with the use of CIs, a 95% CI that does not overlap zero is roughly equivalent to a significant *p*-value in the frequentist's sense [68]; we describe such results as showing 'strong support' for predictions. For estimates that are biased away from zero but whose 95% CIs overlap zero by up to 15%, we use the term 'moderate support'. For estimates centred on zero with 95% CIs greatly overlapping zero we use the term 'no support for an effect' or the term 'strong support for lack of effect'. We used visual assessment of the residuals to evaluate model fit. The strength of correlations was described as either weak ($|r| < 0.3$), moderate ($0.3 < |r| < 0.5$) or strong ($|r| > 0.70$).

3. Results

There was substantial variation in all three traits, both in the average values per individual (i.e. the amount of among-individual variation), and in the range of values expressed per individual (i.e. the amount of within-individual variation). The among-individual variation in body mass ranged from 116 to 186 g, pectoral muscle mass ranged from 24.0 to 33.9 g, and gizzard mass ranged from 4.64 to 8.26 g). As predicted, there was strong support for positive correlations between all these traits at the among-individual level (95% CIs did not overlap with 0), and these correlations were all strong (all $|r| > 0.70$) (table 1, left panel of figure 2). Analyses correcting for the effect of pectoral muscle mass and gizzard mass on total body mass yielded quantitatively similar results (table 1, values in italics), indicating that the among-individual correlations between gizzard mass and overall body mass, and between pectoral muscle mass and overall body mass, were not solely because of part-whole correlations. Additionally, there was moderate (e.g. for gizzard mass and pectoral

muscle mass) to strong support (e.g. for body mass) that all three traits were positively correlated with structural body size (table 2).

The average within-individual variation in body mass was 29 g (range: 4–115 g), pectoral muscle mass was 6.6 g (range 0.8–13.9 g), and gizzard mass was 5.2 (range: 1.4–8.2 g) in the course of the study. As expected, a large proportion of the within-individual variation in gizzard mass could be explained by diet treatment ($R^2 = 45.54\%$). However, the proportion of within-individual variation in pectoral muscle mass and body mass that could be explained by the diet treatment was markedly lower (2.37% and 0.33%, respectively), indicating that additional, non-experimental factors, contributed substantially to the within-individual variation in those traits (e.g. ambient temperature [54]). At the within-individual level, there was strong support for positive correlations between both gizzard mass and body mass, and between pectoral muscle mass and body mass (table 1). The strength of the correlation between body mass and pectoral muscle mass was strong ($|r| > 0.5$), while the strength of the correlation between gizzard mass and body mass was weak ($|r| > 0.10$). We also found moderate support for a weak ($|r| < 0.30$) negative within-individual correlation between gizzard mass and pectoral muscle mass (table 1). When correcting for part-whole correlations, there was strong support for a positive within-individual correlation between pectoral muscle mass and body mass minus pectoral muscle mass of moderate strength. By contrast, there was no support for a correlation between gizzard mass and body mass independent of gizzard mass (table 1).

The results of the RMA regression analyses show that the slope of the relationship between body mass and pectoral muscle mass was quantitatively similar at the among- and within-individual level (see figure 2; electronic supplementary material, table S2). At the among-individual level, a 1 g increase in body mass was associated with a 0.16 g (95% CI: 0.14, 0.19) increase in pectoral muscle mass, while at the within-individual level, 1 g increase in body mass was associated with a 0.21 g (95% CI: 0.20–0.22) increase in pectoral muscle mass. The slope between overall body mass and gizzard mass tended to be steeper at the within-individual level compared with the among-individual level. At the among-individual level, for every 1 g increase in overall body mass, there was a 0.05 g (95% CI: 0.04, 0.06) increase in gizzard mass, compared with 0.17 g (95% CI: 0.15, 0.18) increase at the within-individual level. The slope between gizzard mass

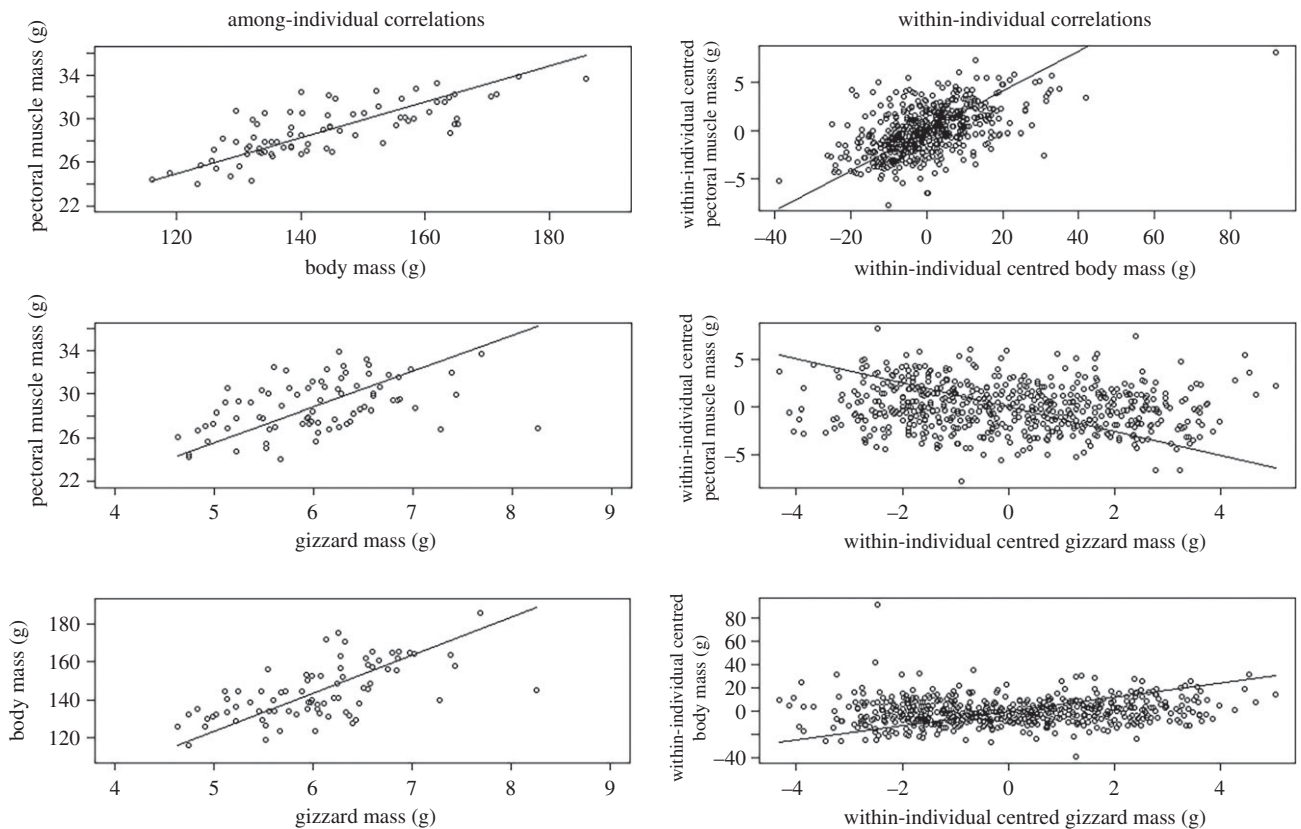


Figure 2. Among-individual (left panel) and within-individual (right panel) correlations between gizzard mass (g), pectoral muscle mass (g) and body mass (g). For the among-individual correlations, each circle represents the average value per individual (mean of between four and eight measurements per individual). For the within-individual correlations, each individual's average trait value was subtracted from each measure, thus, values represent deviations from the individual's average, and each individual is represented by between four and eight data points in each figure in the left panel. Solid lines show the slopes of a reduced major axis (RMA) regression.

Table 2. Results of Pearson's correlations between structural body size (PC1 of wing length, bill length and tarsus length) and average body mass, average gizzard mass and average pectoral muscle mass. (Data from $n = 68$ individuals.)

Pearson's correlation between PC1 and:	r (95% CI)
average body mass	0.33 (0.09, 0.52)
average gizzard mass	0.16 (−0.09, 0.38)
average pectoral muscle mass	0.23 (−0.01, 0.44)

and pectoral muscle mass was of similar magnitude, but in the opposite direction, at the among- versus within-individual level. At the among-individual level, for every 1 g increase in gizzard mass, there was a 3.23 g (95% CI: 2.68, 4.01) increase in pectoral muscle mass, versus a 1.26 g decrease (95% CI: −1.37, −1.16) at the within-individual level.

4. Discussion

We used a multivariate approach to study trait integration at the among- and within-individual level in red knots for three ecologically important traits: body mass, gizzard mass and pectoral muscle mass. Covariation between body mass and pectoral muscle mass, and between gizzard mass and body mass, was positive at both the among- and within-individual levels. Heavier knots had heavier pectoral muscles and gizzards, and

when individual knots become heavier, both their pectoral muscle and gizzard mass increased. However, the relationship between gizzard mass and pectoral muscle mass was level-specific: there was a strongly positive correlation at the among-individual level indicative of trait integration, but a weakly negative correlation at the within-individual level indicative of a trade-off. The negative within-individual covariation between gizzard mass and pectoral muscle mass suggests that these two traits are not independently regulated. We propose that their negative within-individual covariation may be the outcome of an evolved mechanism underlying body remodelling associated with migration.

If body mass, gizzard mass and pectoral muscle mass are all expressions of overall body size, there should be positive among-individual correlations between all three traits. Indeed, in addition to being positively correlated with each other, each of these traits also tended to be positively associated with measures of structural body size (e.g. tarsus length, wing length, bill length; table 2). Structurally larger knots were heavier, had larger gizzards and larger pectoral muscles. However, we also observed positive correlations between gizzard mass and body mass, and between pectoral muscle mass and body mass at the within-individual level, suggesting that associations between these traits occur independently of overall body size (note that structural body size does not vary at the within-individual level, because mature individuals do not change in structural body size [38]).

The positive covariation between total body mass and pectoral muscle mass at both the among- and within-individual levels can be understood from the functional association

that each of these traits has with flight performance [11]. Although the strength of the covariation was slightly lower at the within-individual level (table 1), the estimated increase in pectoral muscle mass per 1 g increase in body mass was quantitatively similar at both the among- and within-individual levels (electronic supplementary material, table S2), suggesting that the covariation between total body mass and pectoral muscle mass is shaped by the same mechanism at both these levels: power output [15].

The covariation between total body mass and gizzard mass was also positive at both the among- and within-individual levels; however, the patterns were quantitatively different. At the among-individual level, the covariation was strong, while at the within-individual level the covariation was weak. We suggest that the differences in the strength of the covariation between gizzard mass and overall body mass across these two levels reflects differences at the within- and among-individual level in the mechanisms generating integration between these traits. Consistent with this interpretation, covariation between body mass after correcting for part-whole correlations (by subtracting the mass of the gizzard) and gizzard mass remained strong and positive at the among-individual level, but disappeared at the within-individual level. We interpret the integration between body mass and gizzard mass at the among-individual level as reflecting body size variation, with larger birds being heavier and also requiring larger digestive organs to fuel their higher energy demands. By contrast, the within-individual correlations can be fully explained by part-whole correlations, suggesting a lack of integration between the traits at this level.

Several empirical studies previously have documented a negative within-individual correlation between gizzard mass and pectoral muscle mass [19,33,42,43]. This negative covariation has been suggested to result from a common factor, migration, exerting opposing effects on two independent traits [31,44,47] and/or limited access to protein, creating a trade-off between investment in gizzard muscle versus pectoral muscle [20,21]. Despite the fact that we conducted the experiments outside the migration periods and under conditions of unlimited access to high protein diets, we observed the within-individual negative covariation between gizzard mass and pectoral muscle mass. Thus, our results demonstrate that neither migration nor protein limitation are required to generate this trade-off. One possible explanation for the apparent trade-off is that the requirements to build up gizzard mass on low digestive quality diets exceeded the capacity to assimilate endogenous protein, which could result in reallocation of pectoral muscle protein even under conditions of high dietary protein. When low digestive quality diets necessitate a rapid build-up of gizzard mass, for every 1 g increase in gizzard mass, knots atrophied their pectoral muscles by 1.3 g (electronic supplementary material, table S2). Previous work has shown that knots can fully adjust their gizzards to changes in the digestive quality of their diets within six days [51]. Given that the knots in the present study had at least three weeks on any diet treatment, this would have allowed two additional weeks to rebuild pectoral muscle mass after adjusting their gizzards. We did not quantify protein assimilation in this study, and therefore, we cannot rule out the possibility that the negative within-individual covariation between gizzard mass and pectoral muscle mass reflects a constraint on protein uptake. However, we suggest this is unlikely to account for our findings

because typical digestion efficiencies of protein by birds are in excess of 50% [72,73]. Furthermore, there was no evidence of protein limitation during our study given that knots maintained good body condition throughout the experiments (K.J. Mathot, E.M.A. Kok 2016, personal observation).

We suggest that an exclusive focus on the functional significance of gizzard mass and pectoral muscle mass is insufficient to understand the observed pattern of covariation. From a functional perspective, in the context of these experiments, there is no reason why developing a larger gizzard would be predicted to favour atrophy of pectoral muscle mass, or *vice versa*. A more complete understanding of patterns of within-individual trait covariances requires explicit consideration of the evolution of the mechanisms mediating trait expression (*sensu* [74]). We suggest that the observed negative within-individual correlation between gizzard mass and pectoral muscle mass may provide insights into the evolved mechanism underlying migration-related body remodelling in red knots.

In free-living knots, a negative within-individual covariation between gizzard mass and pectoral muscle mass is adaptive. During migration, knots benefit from reducing gizzard mass because it does not serve a function during fasting associated with long-distance flights and is costly to maintain, while at the same time they benefit from developing large pectoral muscles to power flights [43]. If large and rapid changes in gizzard mass in free-living knots are reliably associated with conditions that favour simultaneous and opposing changes in pectoral muscle mass, we suggest that this may have led to the evolution of a mechanism whereby large, rapid changes in gizzard mass trigger opposite changes in pectoral muscle mass.

Do large and rapid changes in gizzard mass reliably coincide with migration? Although gizzard mass responds to variation in diet over short time-scales (i.e. approx. one week for twofold adjustment) [51], this magnitude and rate of change has been documented in captive studies in which knots were subjected to large and absolute changes in the quality of their diets (e.g. shift from exclusively high digestive quality diet to exclusively low digestive quality diet). In free-living knots, day to day variation in the quality of encountered prey would neither be as extreme in magnitude, nor as absolute. Even under conditions of starvation, knots defend their gizzard mass at the expense of other lean body components (including pectoral muscle) [17]. Therefore, changes in gizzard mass outside of the migration period are unlikely to be extreme, and the most predictable occurrence of large and rapid changes in gizzard mass are likely to be those observed prior to long-distance flights (e.g. [31,49,74]), when opposing changes in pectoral muscle would indeed be favoured.

We propose that the mechanism underlying migration-related body remodelling operates unidirectionally. That is, large and rapid changes in gizzard mass induce opposing changes in pectoral muscle mass, but not *vice versa*. The reasons for this are twofold. First, this order of organ adjustment is adaptive in the context of migratory remodelling. In free-living shorebirds, gizzard mass atrophy prior to long-distance flights precedes pectoral muscle mass hypertrophy by more than a week [22,43,46]. From an energy management perspective, it seems adaptive to delay the build-up of pectoral muscle mass until shortly before migration given the high metabolic cost of the flight muscles [36], although we cannot exclude the possibility that this pattern reflects an unknown constraint. Second, while large, rapid changes in gizzard mass may be predictably associated with migration (see above), the same

is not true for large and rapid changes in pectoral muscle mass. Pectoral muscle mass decreases rapidly under starvation [17], increases rapidly in response to low temperatures [35,54,75], and closely tracks overall body mass [14]. Thus, rapid changes in pectoral mass cannot serve as a reliable indicator of migration status and therefore cannot be used to direct migration-related changes in gizzard mass. Consistent with this notion, experiments in knots that induced larger pectoral muscles by exposing them to colder ambient conditions found no coincident changes in gizzard mass [54].

Although numerous studies have experimentally generated rapid changes in gizzard mass in knots [51,55,66,76], we are aware of only one other study in which pectoral muscle mass was measured simultaneously. In that study, knots were given a staple diet of high digestive quality following capture, resulting in a large (50%) decrease in gizzard mass [33]. Over the same time period, pectoral muscle mass increased 20%, with almost no change in overall body mass [33]. Although these results are consistent with our proposed mechanism, the timing of gizzard and pectoral muscle measurements, as in the present study, was too coarse to elucidate the timing of changes in pectoral muscle mass relative to gizzard mass. If our proposed mechanism is correct, we would predict that diet-induced changes in gizzard mass precede changes in pectoral muscle mass. This is corroborated by our finding that diet explains a large proportion of the within-individual variation in gizzard mass, while within-individual variation in gizzard mass explains more of the within-individual variation in pectoral muscle mass than does diet. Nonetheless, studies measuring diet-induced changes in gizzard mass and pectoral muscle mass on a finer temporal scale would provide stronger tests of this idea. Further, we would predict that smaller and/or slower changes in gizzard mass (e.g. changes induced by partial changes in diet) would not be sufficient to trigger changes in pectoral muscle. This is because small and/or slow changes in gizzard mass are likely to occur throughout the annual cycle of knots, when coincident changes in pectoral muscle mass are not necessarily beneficial. A mechanism whereby pectoral muscle mass would continuously adjust to small within-individual variations in gizzard mass would therefore not be beneficial.

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5. Conclusion

Many long-distance migrants undergo dramatic body remodelling associated with the acts of migration and being faced by widely different climate and food regimes. Our captive experiments revealed that neither migration nor protein limitation are required to generate the within-individual trade-off between gizzard mass and pectoral muscle mass. We speculate that the negative covariance may provide clues as to the mechanisms coordinating body remodelling in free-living birds. Although our proposed mechanism is speculative and requires empirical scrutiny, this work supports earlier calls for greater integration of function and mechanism within studies, and in particular, the need for more explicit consideration of the evolution of mechanisms [77].

Ethics. These experiments complied with Dutch law and were carried out under permit NIOZ 10.05 addendum 5, and protocol no. AVD802002016740.

Data accessibility. Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.48894fk> [78].

Competing interests. We declare we have no competing interests.

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