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Within-Individual Canalization Contributes to Age-Related Increases in Trait Repeatability: A Longitudinal Experiment in Red Knots

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ABSTRACT: Age-related increases in the repeatable expression of labile phenotypic traits are often assumed to arise from an increase in among-individual variance due to differences in developmental plasticity or by means of state-behavior feedbacks. However, age-related increases in repeatability could also arise from a decrease in within-individual variance as a result of stabilizing trait expression, that is, canalization. Here we describe age-related changes in within-individual and among-individual variance components in two correlated traits—gizzard mass and exploration behavior—in a medium-sized shorebird, the red knot (*Calidris canutus*). Increased repeatability of gizzard mass came about due to an increase in among-individual variance, unrelated to differences in developmental plasticity, together with decreases in within-individual variance consistent with canalization. We also found canalization of exploration but no age-related increase in overall repeatability, which suggests that showing predictable expression of exploration behavior may be advantageous from a very young age onward. Contrasts between juveniles and adults in the first year after their capture provide support for the idea that environmental conditions play a key role in generating among-individual variation in both gizzard mass and exploration behavior. Our study shows that stabilization of traits occurs under constant conditions: with increased exposure to predictable cues, individuals may become more certain in their assessment of the environment allowing traits to become canalized.

Keywords: consistent among-individual differences, variance partitioning, within-individual variation, state-behavior feedbacks.

Introduction

Individuals often differ consistently from one another in suites of behavioral, physiological, and morphological traits (Sih et al. 2004; Bell et al. 2009; Réale et al. 2010a; Dall et al. 2012; Carere and Maestripietri 2013). Although interindividual variability was traditionally viewed as merely the substrate for natural selection, evidence is accumulating that among-individual variation has greater ecological and evolutionary implications (Bolnick et al. 2003; Sih et al. 2012; Wolf and Weissing 2012). It is increasingly recognized that such intraspecific variation may be adaptive (Wilson and Yoshimura 1994; Bolnick et al. 2003; Dall et al. 2004; Sih et al. 2004; Réale et al. 2007).

Individuals of the same population may differ in dispersal behavior (Cote et al. 2010a, 2010b), foraging behavior, resource use (Svanbäck and Bolnick 2007; Toscano et al. 2016; Sheppard et al. 2018), and aggression (Bell et al. 2009). In some cases, these among-individual differences in behavior are associated with differences in reproduction and survival (Wilson 1998; Smith and Blumstein 2008; Réale et al. 2010b). Variation between individuals can lead to nonrandom distributions of individuals, an increase in the range of resources that can be exploited, and higher resilience to change for populations as a whole (Bolnick et al. 2003; Wolf and Weissing 2010; Sih et al. 2012). Therefore, not surprisingly, a significant amount of work has aimed to address the causes of among-individual variation (Wolf et al. 2007; Dingemanse and Wolf 2010).

The ontogeny of repeatable among-individual variation, a potentially core process underlying individual development,

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has received markedly less attention (but see Sinn et al. 2008; Bell et al. 2009; Biro and Stamps 2015; Polverino et al. 2016). Although among individual variance can in theory either increase or decrease with age, the majority of empirical studies have reported age-related increases in repeatable among-individual variation (table 1). However, these studies often fail to evaluate whether these changes are driven by changes in the amount of among-individual variation, within-individual variation, or both (table 1).

Theoretical considerations of the development of trait repeatability have focused on processes that affect the amount of variation among individuals (Sih et al. 2015; Stamps and Frankenhuis 2016; Fisher et al. 2018). One obvious explanation for age-related increases in among-individual trait expression is that it reflects among-individual differences in the conditions experienced during development (West-Eberhard 2003). However, among-individual variation can also arise when individuals are reared under near-identical conditions (Crabbe et al. 1999; Brust et al. 2015; Bierbach et al. 2017). This could be due to (epi)genetic variation among individuals (Dall et al. 2012) or to individual differences in developmental plasticity (i.e., the effect of environment on phenotypic differences; West-Eberhard 1989, 2003; Stamps and Krishnan 2014a; Stamps and Frankenhuis 2016).

Among-individual variance can also increase over time through positive feedbacks between two traits (Sih et al. 2015). For example, foraging boldness (i.e., willingness to forage in the presence of predators) may allow individuals to acquire more resources and grow relatively more quickly compared to individuals that do not forage in the presence of predators (Luttbeg and Sih 2010). If, at the same time, being larger confers some safety advantage (e.g., because predators are gape limited and large prey are less accessible), then being larger will also favor higher boldness (Luttbeg and Sih 2010). The reciprocal effects of boldness on body size and body size on boldness mean that initially very small differences between individuals can increase over time (Sih et al. 2015).

Though less often considered, increased repeatability during development may also result from decreasing within-individual variance, or canalization (Waddington 1942). A trait is considered canalized if phenotypic expression remains invariable under mildly differing developmental conditions. The term canalization was originally used to refer to the evolution of developmental stability (Waddington 1942). According to Waddington's (1942) metaphor of canalization, the individual development of traits can be visualized as the movement of balls rolling down alternative valleys in a landscape that has been shaped by natural selection. Environmental effects can be implemented either as modifications to the width and depth of a single valley (Boonekamp et al. 2018) or as switches between alternative valleys (Waddington 1942). More recently, the term canalization has been applied to refer to the reduction in residual phenotypic variance at the within-

individual level (Westneat et al. 2015). A reduction in within-individual variance (i.e., residual variance) can occur if phenotypic variation decreases in the course of development (e.g., Stamps and Krishnan 2014b, 2017; Westneat et al. 2015; Stamps and Frankenhuis 2016). Following Waddington's metaphor, this is analogous to the valleys in the phenotypic landscape to deepen and/or narrow over time, producing more rigid and predictable trait expression across ontogeny (Boonekamp et al. 2018).

Thus, age-related increases in trait repeatability under identical conditions can be the outcome of at least three distinct developmental processes. Two of these affect the degree of among-individual variance (i.e., differences in developmental plasticity and state-behavior feedbacks), and one affects the degree of within-individual variance (within-individual canalization; for a matrix of predictions, see table 2; fig. A1; figs. A1, A2 are available online). Recognizing that many organisms are only sensitive to environmental cues during particular stages of ontogeny (Bateson 1979; Fawcett and Frankenhuis 2015; Panchanathan and Frankenhuis 2016), systematic investigation of the development of trait expression requires longitudinal studies of development.

We studied the development of two ecologically important phenotypic traits in a migratory shorebird: gizzard mass (Piersma et al. 2003; van Gils et al. 2003, 2005) and exploration behavior (Bijleveld et al. 2014, 2016; Oudman et al. 2016). In a longitudinal study spanning two consecutive years, we measured individual changes in gizzard mass and exploration behavior in red knots (*Calidris canutus*; hereafter called "knot"). During the nonbreeding season, knots forage on a diet of hard-shelled prey, primarily mollusks, that they crush in their muscular gizzards (Zwarts and Blomert 1992; Piersma et al. 1993; Battley and Piersma 2005), and gizzard mass is therefore a key trait (van Gils et al. 2005). Further, exploration behavior scored in standardized behavioral assays (see "Material and Methods") has been shown to be correlated with large-scale (hundreds of kilometers) patterns of space use in the wild (Bijleveld et al. 2014). Notably, exploration and gizzard mass in free-living knots covary at the among-individual level; individuals with large gizzards at the time of capture have lower exploration scores than individuals with small gizzards (Bijleveld et al. 2014, 2016). Previous laboratory experiments have shown that both gizzard mass and exploration behavior exhibit repeatable among-individual variation in knots (≥ 2 calendar year; see "Material and Methods" for explanation; Bijleveld et al. 2014; Mathot et al. 2017). However, experimental manipulations of gizzard mass produced no changes in exploration behavior (Bijleveld et al. 2014). These series of observations led to the speculation that the among-individual variation in gizzard mass and exploration behavior could be the result of state-behavior feedbacks between searching behavior and digestive quality of food during a limited window in early ontogeny (Bijleveld

et al. 2014). Here we describe the development of age-related difference in trait repeatability in the light of three nonexclusive developmental processes to elucidate the developmental origin of among-individual variation in trait expression.

Material and Methods

Study Species and Housing Conditions

The knots (*islandica* subspecies; Piersma 2007) used in this study were captured with mist nets at two different high-tide roosts in the Dutch Wadden Sea—Schiermonnikoog (53.29°N, 6.15°E; $n = 53$) and Griend (53.15°N, 5.16°E; $n = 31$)—between August 20 and October 20, 2015. Birds were aged based on plumage characteristics and classified as either juvenile (<6 months), in their second calendar year (6–18 months), or older (i.e., adult birds, >18 months; Prater et al. 1977). Only juveniles and adults were selected for the study ($N = 44$ juveniles, $N = 46$ adults). We collected a small blood sample (<75 μ L) for molecular sexing (van der Velde et al. 2017). For simplicity, we refer to the birds caught as first-year birds as “juveniles” throughout the article, despite the fact that they changed from being juvenile to second calendar year to adult in the course of this 2-year-long study.

Birds were housed in outdoor aviaries (4.0 m deep, 1.9 m wide, and 2.3 m high at one end, sloping down to a height of 1.9 m across the depth of the aviary) at the Experimental Shorebird Facility of the NIOZ Royal Netherlands Institute for Sea Research on the island of Texel, Netherlands (53°00'N, 04°47'E). The aviaries had smoothly coated concrete floors that were constantly irrigated with running seawater. The back of each aviary had a basin with sand collected from the Wadden Sea and running seawater. Outside of experiments, birds had ad lib. access to Trout food pellets (Produits Trouw, Vervins, France) and a continuous source of fresh water for drinking and bathing in a separate tray. Every week, while the aviary floors were cleaned and disinfected with chlorine, the birds were weighed, their molt and plumage status scored, and their bodies, especially their feet, checked for small wounds and *Staphylococcus* infection (Milot et al. 2014).

The focal birds (*islandica* subspecies) were kept together with knots of the *canutus* subspecies in mixed flocks (14–17 knots per aviary, randomized with stratification based on age and subspecies). Flock composition was largely constant throughout the first year, but before the start of the second year of experiments new birds of the *canutus* subspecies were caught ($N = 22$). Thus, to maintain constant flock sizes across the two study years, 24 *islandica* knots were released between year 1 and year 2 of the experiment.

Diet Manipulations

To prevent circannual endogenous rhythms from unduly affecting our measurements (Battley and Piersma 2005, Ka-

ragicheva et al. 2016), experiments were only carried out over two nonbreeding periods, from late October 2015 to early April 2016 in year 1 and from early October 2016 to mid-March 2017 in year 2. During the experimental period, birds were fed ad lib. diets of either high or low digestive quality. The high-digestive-quality food (HQ) consisted of Trout food pellets, and the low-digestive-quality (LQ) food was thawed mud snails, *Peringia ulvae*. Previous work has shown that these two food types induce approximately twofold variation in gizzard mass (Vézina et al. 2011; Mathot et al. 2017). We used a crossover design: birds in half of the aviaries ($n = 4$) were fed HQ food first, while the other half received LQ food first. Previous studies showed that knots can fully adjust their gizzard mass to a new food type within approximately 1 week (Dekinga et al. 2001), but we allowed 3 weeks of acclimatization to the new diet to ensure that the general condition of the birds would be stable and equal between diets (Bijleveld et al. 2014; Mathot et al. 2017).

These 3 weeks of diet manipulation were followed by 2 weeks of behavioral observation, during which time the knots remained on the same ad lib. diet. When all behavioral observations were completed, a new replicate of diet manipulations commenced; aviaries previously assigned the HQ food treatment became LQ aviaries, and vice versa. Four diet treatments were carried out per bird during each of the two experimental years. To prevent systematic differences between the knots resulting from the order of testing, we randomized the sequence with which we tested individuals in each behavioral test. On average, 43 days (ranging from 21 to 65 days) elapsed between successive behavioral tests in year 1 and 40 days (ranging from 24 to 57 days) between successive tests in year 2.

Gizzard Mass Measurements

After each diet treatment, and before behavioral observations, the gizzard mass of all birds was measured using ultrasonography (Dietz et al. 1999; Dekinga et al. 2001). To standardize the measurements, birds were deprived of food for at least 1 h prior to measurement to ensure an empty gizzard. Subsequently, birds were selected in a haphazard order for measurement. The observer was blind to the age and diet treatment of the birds. Gizzard measurements were done following a standardized procedure developed by A. Dekinga; see Mathot et al. (2017) for a detailed description of the method.

Exploration Behavior

The exploration behavior of individual birds was quantified in an arena that was novel for the birds during first exposure. Studies on exploration traditionally focus on individual movements after introduction to a novel environment (Verbeek et al. 1994; Réale et al. 2007). Studies that assess

Table 1: Nonexhaustive review of articles reporting age-related changes in trait repeatability

Class, species	Age group	Trait	Age-related effect	R/VC	Reference
Insects:					
Red flour beetle (<i>Tribolium castaneum</i>)	Subadult→young adult→adult	B: Movement B: Edge preference	– –	R R	Wexler et al. 2016
Wild cricket (<i>Gryllus campestris</i>)	Juvenile→adult	B: Flight initiation distance	–	VC	Niemelä and Dingemanse 2017
Speckled wood butterfly (<i>Pararge aegeria</i>)	Juvenile→adult	B: Activity ^a	– ^b	VC	Kaiser et al. 2018
Arachnids:					
Desert funnel-web spider (<i>Agelenopsis lisa</i>)	Juvenile→penultimate molt→sexually mature	B: Foraging B: Exploration B: Neophobia B: Boldness	↑ ↑/↓ ^c – (M), ↑ (F) ↑ (M), – (F)	R R R R	Bosco et al. 2017
Basal tarantula (<i>Brachypelma smithi</i>)	Juvenile (1 year old)→immature (2 years old)	B: Boldness B: Latency to attack B: Conspecific tolerance B: Neophobia	– ^c – ^c ↑ ^c – ^c	R R R R	Bengston et al. 2014
Fish:					
Eastern mosquitofish (<i>Gambusia holbrooki</i>)	Juvenile→subadult→adult	B: Distance moved B: Freezing time B: Hiding time B: Latency to emerge	↑ ↑ ↑ ↑	R R R R	Polverino et al. 2016
Amazon molly (<i>Poecilia formosa</i>)	Hatchling→juvenile	B: Movement	↑	VC	Bierbach et al. 2017
White shark (<i>Carcharodon carcharias</i>)	Subadult→adult	B: Prey choice	↑	VC	Kim et al. 2012
Crayfish (<i>Cherax destructor</i>)	0→4 months	P: Growth rate B: Boldness	↑ ↑	R	Biro et al. 2014

Reptiles:									
Western fence lizard (<i>Sceloporus occidentalis</i>)	Hatchling→juvenile→subadult				B: Locomotion performance M: Body size			R	Van Berkum et al. 1989
Gecko (<i>Lepidodactylus lugubris</i>)	Juvenile→adult				B: Exploration B: Boldness			VC VC	Sakai 2018
Birds:									
Zebra finch (<i>Taeniopygia guttata</i>)	Subadult→young adult→adult				B: Fearlessness B: Exploration B: Activity B: Aggression B: Boldness			R R R R R	Wuerz and Krüger 2015
House sparrow (<i>Passer domesticus</i>)	Nestling→adult				P: Corticosterone levels			R	Lendvai et al. 2015
Mammals:									
Yellow-bellied marmot (<i>Marmota flaviventris</i>)	Juvenile→yearling→adult				B: Boldness B: Docility			R R	Petelle et al. 2013
Eurasian harvest mice (<i>Micromys minutus</i>)	Juvenile→adult				B: Exploration B: Activity B: Boldness C: Spatial recognition			R R R R	Schuster et al. 2017
European roe deer (<i>Capreolus capreolus</i>)	Juvenile/adult				B: Docility			R	Debeffe et al. 2015

Note: In each study, age groups were compared using either a longitudinal approach (right arrow; i.e., measuring the same individual over different life stages), a cross-sectional approach (backslash; i.e., comparing individuals belonging to different life stages), or a combination of both approaches. The type of trait is indicated as B (behavioral), M (morphological), P (physiological), or C (cognitive), with a brief description. Age-related effects are summarized as increasing repeatability (up arrow), no change (dash), or decreasing repeatability (down arrow). In situations where effect differed between sexes, this is indicated with M (males) and F (females). The column labeled R/VC indicates whether only changes in repeatability (R) were considered or changes in within-individual and among-individual variance components (VC) were assessed (or provided) separately.

^a Note that other behaviors were also scored in this study, but the only common assay across age cohorts was activity.

^b Did not formally compare the repeatability across the age classes, but point estimates were presented and could be compared.

^c Repeatability increased from juvenile to penultimate molt and decreased from penultimate molt to mature adult.

^d Juvenile and adult marmots showed no repeatability in boldness, but yearlings did.

^e Boldness is repeatable in juveniles and in adult males but not in females. This implies an age-related decrease in repeatability for females. But this is not explicitly discussed in the article.

Table 2: Predicted alternative processes that may generate age-related increases in trait repeatability

Process	Among-individual variance	Within-individual variance	Within-individual trait covariance
Individual differences in developmental plasticity	↑	—	N ^a
State-behavior feedbacks	↑ ^b	—	Y ^c
Within-individual canalization	—	↓	N

Note: Predicted age-related changes in among-individual and within-individual variances (up arrow = increase; down arrow = decrease; dash = no prediction) and the presence of within-individual trait covariances (Y = Yes; N = No) for three alternative processes that may generate age-related increases in trait repeatability. Predicted age-related changes in among-individual variance and within-individual variance apply both within individuals over time as well as across age categories (e.g., between juveniles vs. adults).

^a Within-individual trait covariance is not explicitly predicted from individual differences in developmental plasticity. However, when developmental plasticity of two traits covaries, this could generate within-individual trait covariance.

^b Our experimental protocol interrupts potential state-behavior feedbacks (see “Material and Methods”). Thus, we would not predict increased among-individual variance within individuals over time. However, we would expect to find lower among-individual variance in juveniles than in adults in year 1, as knots captured as adults would have experienced state-behavior feedbacks prior to capture.

^c Within-individual trait covariance is present during ontogeny and absent after ontogeny.

repeatability of exploration typically reuse the same test arena for subsequent tests (e.g., Dingemanse et al. 2002, 2012; Minderman et al. 2010; Bijleveld et al. 2014; McCowan et al. 2015; Wuerz and Krüger 2015; Dubuc-Messier et al. 2017). Therefore, a decline in novelty with repeated exposure is an inherent feature of studies estimating repeatability in exploration. The exploration arena used here was identical to the one used in Bijleveld et al. (2014). It measured 7 × 7 m and was filled with a layer of 30 cm seawater and five 1 × 1-m trays filled with wet sand (fig. A2).

Birds were caught from their holding aviaries 2 h prior to their randomly assigned observation time and kept individually in holding crates in a semidark and quiet room. Birds were food deprived during these 2 h to standardize hunger levels between birds. Immediately prior to the test, each bird was moved to a small aviary adjacent to the arena. After 5 min of acclimatization, the door between the aviary and the arena was opened by means of a remote pulley system, and the bird was gently herded into the arena. Exploration trials lasted 30 min, during which time the behavior of the bird was scored live through one-way glass using the behavioral observation software JWatcher (<http://www.jwatcher.ucla.edu/>) and recorded (GoPro HERO+ LCD) for future reference. The observations were done by five different observers (three observers in year 1 and four observers in year 2, with one common observer across both years), who were randomly distributed over the trials and blind to the treatment and age of the birds being tested. Behaviors recorded were as follows: flying, walking on patches, searching for food, preening, resting, vigilance, out of sight, or other. After the trial ended, the bird was returned to its holding aviary.

Using the protocol developed by Bijleveld et al. (2014) for the same subspecies of red knot, exploration behavior was scored as the fraction of time spent searching or walking on patches. Proportions were logit transformed to meet normality assumptions (Warton and Hui, 2011). This measure of exploration may not be assaying information gathering by

individuals in a broad sense but more specifically information gathering related to the distribution of food.

All experiments described in this article complied with Dutch law and regulation and were carried out under protocol AVD802002016740.

Data Selection and Statistical Analyses

We obtained 569 complete observations of exploration behavior and gizzard mass ($N_{\text{adult}} = 283$, $N_{\text{juvenile}} = 286$). We excluded replicates during which the exploration experiments were disturbed ($n = 2$), when birds swam in the arena for more than 10 consecutive minutes ($n = 11$) because of welfare concerns for the bird (i.e., the risk of drowning or inability to thermoregulate with wet feathers), or when the diet manipulation was unsuccessful ($n = 14$). Unsuccessful diet manipulations occurred when knots failed to switch to the experimentally determined diet (as evidenced by significant body mass loss) or when knots had to be removed from the experiment to be treated for *Staphylococcus* infection.

We constructed univariate models for gizzard mass and exploration behavior to study the development of age-related difference in trait repeatability. To be able to compare both age groups between year 1 and year 2, as well as to contrast adults and juveniles, we constructed separate models for each age group in each year (i.e., four models per trait: juveniles year 1, juveniles year 2, adults year 1, adults year 2). Because we were explicitly interested in age- and year-specific estimates for both among-individual and within-individual variance components, we included a random intercept for individual ID.

Although contrasts between the among-individual and within-individual variance components for each age cohort and year combination could have been carried out in a single analysis by modeling heterogeneous residual errors, such analyses have very low statistical power (Cleasby and Nakagawa 2011). Therefore, we split the data in four bins

and estimated the variance components for each trait per age group and year. To be able to correctly calculate within-individual variance that was unrelated to diet, we used two measures of gizzard mass and exploration on each diet per year, following Araya-Ajoy et al. (2015). We obtained complete data records (i.e., two measures of gizzard mass and exploration on each diet in each year ($N_{\text{replicates per bird}} = 8$) for a total of 58 birds (30 adults and 28 juveniles; $N_{\text{adult measurements}} = 240$, $N_{\text{juvenile measurements}} = 224$). This data can be found in the Dryad Digital Repository (<https://dx.doi.org/10.5061/dryad.dn28cn6>; Kok et al. 2019). We restricted our analyses to these birds, as any changes in variance components from year 1 to year 2 necessarily reflected changes in variance components within individuals or due to age or time in captivity, as opposed to changes resulting from comparing different cohorts of birds. However, our data selection criteria did not affect the estimates of either the fixed effects or the variance components (results not shown).

We modeled gizzard mass and logit exploration as a function of sex (two-level factor: M or F), diet (two-level factor: LQ or HQ), replicate (continuous factor: range 1–4), and the interaction between diet and replicate. The addition of replicate in the model allowed us to test for changes in the response variables over time. The interaction term between diet and replicate allowed for a comparison of diet-related differences in the effect of replicate. In the results section, we focus on the effects of diet, replicate (time), and their interaction on gizzard mass and exploration. We did, however, also include a fixed effect for sex to control for potential differences due to structural size differences between the sexes, since female knots are larger than males (Tomkovich 1992),

but we will not discuss this any further in the results. For the models of exploration behavior, we fitted an additional random intercept for observer ID to control for potential among-observer differences in behavioral scoring that would otherwise introduce additional residual variance. Because the observer was blind to the age group and diet treatment of each experimental bird and because birds were randomly assigned to each observer, observer effects are not biologically meaningful and are not relevant for the hypotheses being tested. They are presented in table 3 for completeness but are not discussed further. Models were built using the lmer function from the lme4 package (Bates et al. 2015) in the R (ver. 3.4.3) statistical environment (R Core Development Team 2017).

We report adjusted repeatabilities (i.e., after correcting for the fixed effects in the model) that were calculated following Nakagawa and Schielzeth (2010, 2013). To study the age-dependent changes in repeatabilities, we first compare the changes in repeatability of gizzard mass and exploration for juveniles and adults between year 1 and year 2 in a longitudinal analysis. Subsequently, we report cross-sectional comparisons (e.g., comparing juveniles in year 1 with adults in year 1 as well as juveniles in year 2 with adults in year 2) to separate age-dependent effects from effects resulting from free-ranging experience or time in captivity. In all cases, we report how both within-individual (i.e., residual) and among-individual variance components contributed to the overall repeatability (Cleasby and Nakagawa 2011).

In cases where we found a value of zero for the among-individual variance, we verified that this was not a false negative result (e.g., singularity due to model overfitting) by

Table 3: Sources of variation in logit-transformed exploration behavior

	Juveniles		Adults	
	Year 1	Year 2	Year 1	Year 2
Fixed effects:	$\beta \pm 95\% \text{ CI}$	$\beta \pm 95\% \text{ CI}$	$\beta \pm 95\% \text{ CI}$	$\beta \pm 95\% \text{ CI}$
Intercept: ^a	-.92 (-1.86, .04)	-1.67 (-2.89, -.27)	-.98 (-1.87, .35)	-1.37 (-2.99, .18)
Diet (LQ)	-.08 (-1.18, .76)	.30 (-1.63, 2.10)	.39 (-.55, 1.44)	.68 (-1.54, 2.32)
Sex (M)	.14 (-.57, .97)	.56 (-.20, 1.27)	-.14 (-1.11, .75)	-.61 (-1.19, .35)
Replicate	-.14 (-.40, .14)	.00 (-.12, .26)	-.34 (-.60, -.09)*	.12 (-.13, .26)
Diet (LQ):replicate	.14 (-.21, .50)	-.01 (-.30, .27)	-.20 (-.53, .20)	-.07 (-.37, .22)
Random effects:	$\sigma \pm 95\% \text{ CI}$	$\sigma \pm 95\% \text{ CI}$	$\sigma \pm 95\% \text{ CI}$	$\sigma \pm 95\% \text{ CI}$
Bird ID	.86 (.57, 1.18)	.70 (.55, 1.11)	1.23 (.91, 1.70)	.90 (.63, 1.20)
Observer	.02 (.00, .13)	.00 (.00, .00)	.15 (.01, .13)	.00 (.00, .00)
Residual variance	1.02 (.75, 1.26)	.61 (.46, .80)	1.12 (.84, 1.42)	.69 (.54, .92)
Repeatability:	$r \pm 95\% \text{ CI}$	$r \pm 95\% \text{ CI}$	$r \pm 95\% \text{ CI}$	$r \pm 95\% \text{ CI}$
Bird ID	.48 (.37, .57)	.60 (.46, .66)	.53 (.44, .62)	.55 (.46, .64)

Note: Exploration behavior is defined as the fraction of time searching.

^a Intercept estimated for females on a high-quality diet during their first replicate.

* $p < .006$ (i.e., 95% credible interval [CI] does not overlap zero).

rerunning the model with bird ID fitted as a fixed effect rather than a random effect and evaluating its effect size. We found no evidence that any of our estimates of zero among-individual variance were due to model overfitting (results not shown).

We used the `sim` function of the `arm` package (Gelman and Su 2016) to simulate values of the posterior distribution of the model parameters (Gelman and Hill 2007). We then extracted 95% credible intervals (CIs) around the mean (β) based on 1,000 simulations using the `MCMCglmm` package (Hadfield 2010). The 95% CI indicates a margin of error in terms of a range of plausible values for β . With this CI, we indicate that we are 95% confident that our CI includes the actual effect size (Cumming and Finch 2005). To evaluate the differences between means and CIs of the fixed effects, as well as the variance components and the repeatability estimates, we followed Cumming and Finch (2005). Independent 95% CIs were deemed to indicate significant differences between averages when they did not overlap. This corresponds to a traditional p value of $<.006$. In cases where the 95% CIs did overlap, we used the proportion of overlap of the 95% CIs (the overlap between the two independent CIs divided by the average CI) to calculate the exact probability (p) that two CIs overlap (Cumming 2009) and discuss the level of support for a given effect as a continuous rather than binary characteristic.

Results

Changes in Mean Trait Values

In each age group in both years, knots developed heavier gizzards on the LQ diet treatment than on the HQ diet treatment (95% CIs for fixed effect diet do not overlap with

0, except for a 0.15 proportion of overlap in juveniles in year 2; table 4). Within each year, diet effects were similar between juveniles and adults, indicating similar gizzard mass plasticity across age cohorts. With time, the relatively light gizzard mass as a result of the HQ diet increased for both juveniles and adults (in year 2, the 95% CIs for replicate do not overlap with 0; table 4). The negative interaction effect between diet and replicate found for adults in year 1 showed a decrease in gizzard masses on the LQ diet treatment across replicates (table 4). These contrasting effects of replicate on HQ versus LQ diet meant that the overall diet effect decreased across replicates (fig. 1).

Exploration did not differ significantly between years or across age groups (all 95% CIs overlap), but exploration behavior decreased across replicates in adults in year 1 (95% CI for replicate does not overlap with 0; table 3). Contrary to expectation, we found no effect of diet on exploration in any of the age groups (all 95% CI overlap with 0; table 3; fig. 2).

Age-Dependent Changes in Trait Repeatability

Juvenile knots showed no repeatability in gizzard mass in year 1 (r and 95% CI centered on 0) and a small but significant repeatability in year 2 (95% CI does not overlap with 0; table 4; fig. 3). In contrast, juvenile knots already showed significant repeatability in exploration behavior in year 1 (95% CI does not overlap with 0), and there was no support for change in the repeatability between year 1 and year 2 ($p = .29$; table 3; fig. 3).

In adults, we observed significant repeatability in gizzard mass in both year 1 and year 2 (95% CIs do not overlap with 0). However, the repeatability in year 2 was significantly lower

Table 4: Sources of variation gizzard mass (g)

	Juveniles		Adults	
	Year 1	Year 2	Year 1	Year 2
Fixed effects:	$\beta \pm 95\% \text{ CI}$	$\beta \pm 95\% \text{ CI}$	$\beta \pm 95\% \text{ CI}$	$\beta \pm 95\% \text{ CI}$
Intercept: ^a	4.50 (3.22, 5.42)	2.42 (.81, 5.06)	4.04 (3.12, 4.91)	2.03 (-.20, 3.85)
Diet (LQ)	4.13 (2.84, 5.72)*	1.97 (-.88, 4.96)	4.86 (3.78, 6.06)*	4.23 (1.44, 7.21)*
Sex (M)	-.56 (-1.16, -.07)*	-1.02 (-1.53, -.56)*	-.64 (-1.20, -.13)*	-.90 (-1.31, -.31)*
Replicate	.38 (-.06, .70)	.46 (.10, .73)*	.26 (.00, .58)	.44 (.16, .76)*
Diet (LQ):replicate	-.29 (-.88, .17)	.05 (-.44, .44)	-.90 (-1.19, -.34)*	-.44 (-.74, .13)
Random effects:	$\sigma \pm 95\% \text{ CI}$	$\sigma \pm 95\% \text{ CI}$	$\sigma \pm 95\% \text{ CI}$	$\sigma \pm 95\% \text{ CI}$
Bird ID	.00 (.00, .00)	.08 (.04, .13)	.11 (.07, .20)	.03 (.02, .05)
Residual	2.60 (1.91, 3.14)	1.77 (1.30, 2.14)	1.67 (1.27, 2.08)	1.82 (1.38, 2.27)
Repeatability:	$r \pm 95\% \text{ CI}$	$r \pm 95\% \text{ CI}$	$r \pm 95\% \text{ CI}$	$r \pm 95\% \text{ CI}$
Bird ID	.00 (.00, .00)	.05 (.03, .07)	.07 (.04, .11)	.02 (.01, .03)

^a Intercept estimated for females on a high-quality diet during their first replicate.

* $p < .006$ (i.e., 95% credible interval [CI] does not overlap zero).

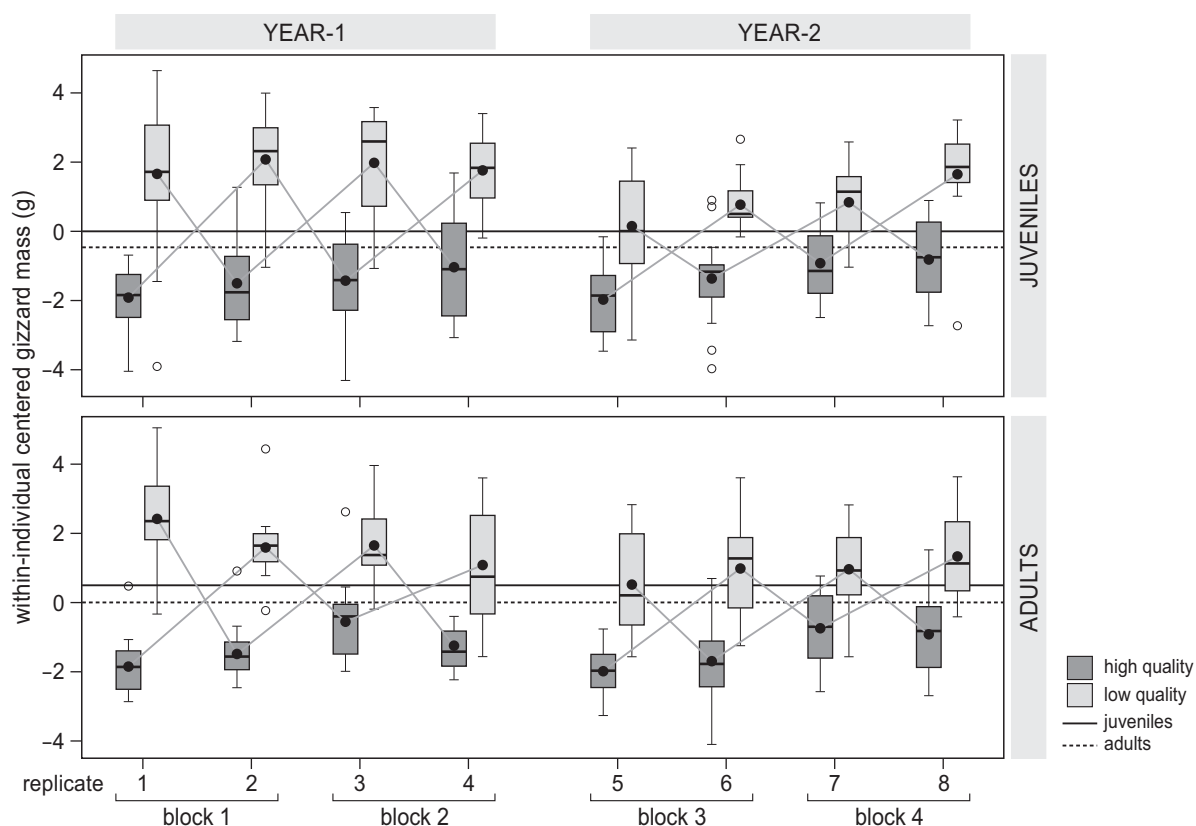


Figure 1: Gizzard mass as a function of manipulated diet and time. Boxes represent the median, quartiles, and interquartile outliers in within-individual-centered gizzard mass (g) for juveniles (*top*) and adults (*bottom*). Gizzard mass was centered within individuals by subtracting the individual’s mean from each measurement ($n = 8$). Dark gray indicates birds on high-quality food (pellets), and light gray indicates birds on low-quality food (mudsnails). Means (filled circles) are connected with gray lines for each group of individuals receiving the similar treatment order in the crossover design. The horizontal black lines represent the mean gizzard mass for juveniles (continuous line) and for adults (interrupted line). Measurements taken during the nonbreeding season in year 1 (i.e., replicates 1–4) are separated from measurements taken during the nonbreeding season in year 2 (i.e., replicates 5–8) by a breeding summer when no measurements were taken.

(95% CIs do not overlap; table 4; fig. 3). For exploration behavior in adults, we found significant (95% CIs do not overlap with 0) and near identical repeatabilities in year 1 and year 2 (proportion overlap between years: $p = .89$; table 3; fig. 3).

When comparing gizzard mass repeatabilities between age groups, repeatability was lower in juveniles than in adults in year 1 (95% CIs do not overlap). In year 2, both repeatability estimates were very small but juvenile repeatability was higher when compared to adults ($p < .01$; table 4; fig. 3). For exploration behavior, we found no differences in repeatability between adults and juveniles in year 1 ($p = .54$) or in year 2 ($p = .90$; table 3; fig. 3).

Changes in Among-Individual and Within-Individual Variance Components

In juveniles, the increase in gizzard mass repeatability between year 1 and year 2 was the result of an increase in among-individual variance (95% CIs do not overlap), to-

gether with a decrease in within-individual variance of 32% between year 1 and year 2 ($p = .03$; table 4; fig. 3). For exploration behavior, we found no significant change in repeatability between year 1 and year 2. However, in the absence of a change in among-individual variance in juveniles ($p = .90$; table 3; fig. 3), a significant decrease in within-individual variance ($p < .01$) led to a (nonsignificant) increase in repeatability.

In adults, the significant decrease of gizzard mass repeatability between year 1 and year 2 was the result of a decrease in among-individual variation (95% CIs do not overlap), while the within-individual variance did not change ($p = .90$; table 4; fig. 3). We found no change in repeatability in exploration behavior for adults from year 1 to year 2. However, when considering the changes in among-individual and within-individual variance components separately, we found a decrease in the within-individual variance ($p = .01$), together with a nonsignificant concomitant decrease in among-individual variance ($p = .15$; table 3; fig. 3).

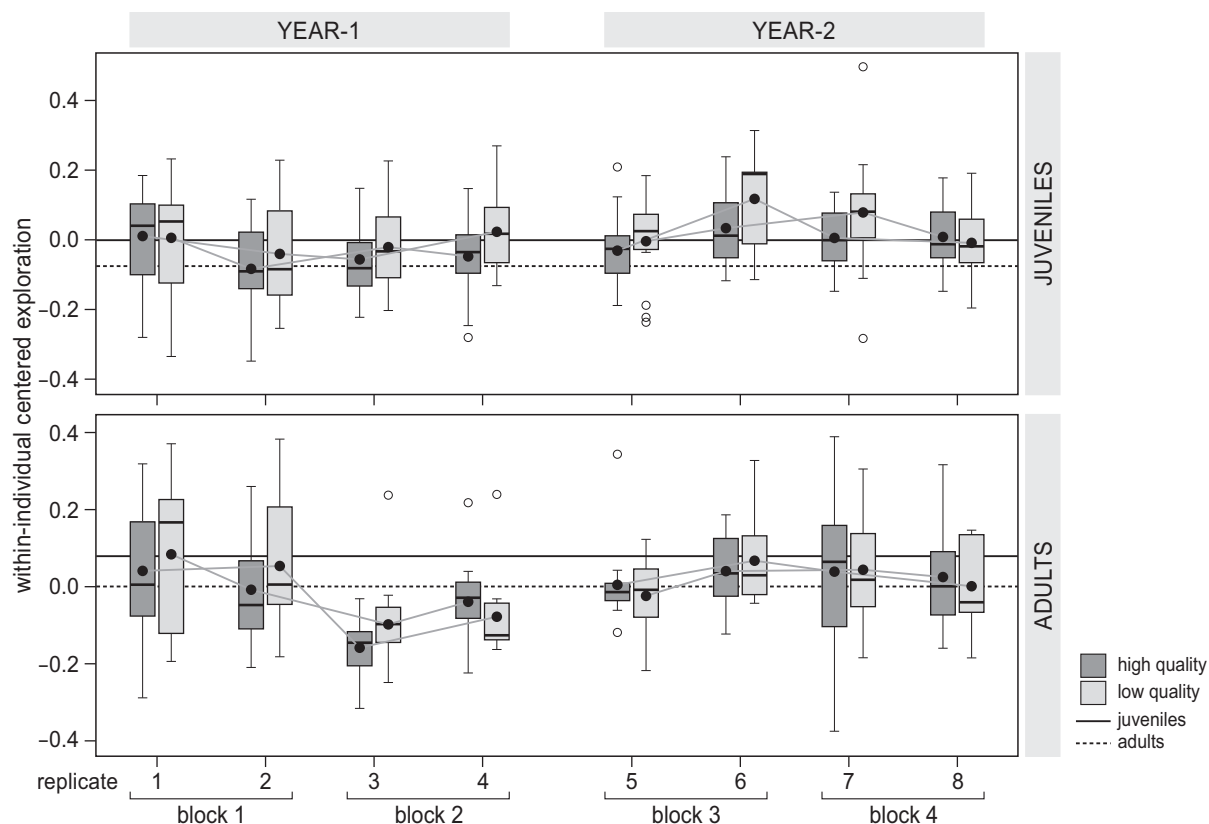


Figure 2: Exploration behavior scores as a function of manipulated diet and time. Boxes represent the median, quartiles, and interquartile outliers in within-individual-centered scores for exploration behavior for juveniles (*top*) and adults (*bottom*). Exploration was centered within individuals by subtracting the individual's mean from each measurement ($n = 8$). Dark gray indicates birds on high-quality food (pellets), and light gray indicates birds on low-quality food (mudsnails). Means (filled circles) are connected with gray lines for each group of individuals receiving the similar treatment order in the crossover design. The horizontal black lines represent the mean exploration behavior for juveniles (continuous line) and for adults (dashed line). Measurements taken during the nonbreeding season in year 1 (i.e., replicates 1–4) are separated from measurements taken during the nonbreeding season in year 2 (i.e., replicates 5–8) by a breeding summer when no measurements were taken.

The age-related difference in gizzard mass repeatability between juveniles and adults in year 1 was due to lower among-individual variance and higher within-individual variance in juveniles compared with adults (among-individual variance: 95% CIs do not overlap; within-individual variance: $p = .01$; table 4; fig. 3). In year 2, the small but significantly higher repeatability in gizzard mass of juveniles compared to adults was the result of higher among-individual variance in juveniles than in adults ($p = .01$). The within-individual variance did not differ between juveniles and adults ($p = .85$; table 4; fig. 3).

As for repeatability, the within-individual ($p = .73$) and among-individual ($p = .11$) variance in exploration behavior did not differ between juveniles and adults in year 1 (table 3; fig. 3). However, the limited overlap in 95% CIs in among-individual variance in adults and juveniles suggests that adults showed higher among-individual variance than juveniles in year 1 (table 3; fig. 3). In year 2, there was no dif-

ference between adults and juveniles in either the within-individual ($p = .63$) or among-individual ($p = .92$) variance in exploration behavior (table 3; fig. 3).

Discussion

In this study, we evaluated support for three nonexclusive developmental processes that may underlie age-related changes in repeatable trait expression in knots (individual difference in phenotypic plasticity, state-behavior feedbacks, and within-individual canalization; table 2). From year 1 to year 2, gizzard mass repeatability increased in juveniles and decreased in adults. This increase in gizzard mass repeatability in juveniles was the result of an increase in among-individual variance and a decrease in within-individual variance. In adults, the decrease in repeatability was due to a decrease in among-individual variance alone. Initially (in year 1), juveniles showed lower among-individual variance

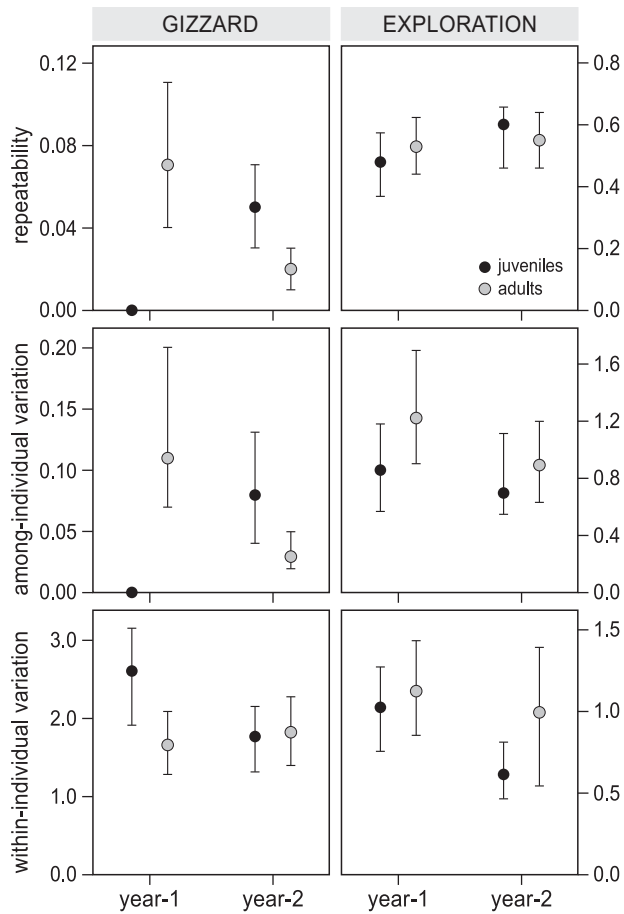


Figure 3: Development of variance components of gizzard mass and exploration behavior. Adjusted repeatabilities (*top*), among-individual variation (*middle*), and within-individual variation (*bottom*) for gizzard mass (*left*) and exploration behavior (*right*) for juveniles (black) and adults (gray) in year 1 and year 2. Circles and bars represent the mean (β) plus 95% credible interval for each age group per year.

and higher within-individual variance in gizzard mass than adults. In year 2, within-individual variance in juveniles declined to levels similar to older individuals (i.e., adults in year 1 and year 2). We found no linear age-related changes in among-individual variance in gizzard mass. Although exploration repeatability did not differ between age groups and years, we found a significant decrease in within-individual variance for both juveniles and adults between year 1 and year 2.

The observed age-related differences in gizzard mass repeatability resulted from changes in both the among-individual and within-individual variance components (table 4). We can exclude the possibility that feedbacks led to an increase in gizzard mass repeatability, because state-behavior feedbacks would have presented themselves as within-individual correlations between gizzard mass and exploration (Luttbeg and Sih 2010; Sih et al. 2015), and we

found nothing to support this (table 2, process 2; table A1, available online). We considered the possibility that the observed increase in among-individual difference in gizzard mass in juveniles between year 1 and year 2 came about as a result of individual differences in developmental plasticity (table 2, process 1). However, since we found a decrease in among-individual variance in gizzard mass in adults between year 1 and year 2, we do not interpret these—apparently reversible—changes in among-individual variance in gizzard mass as the outcome of individual differences in developmental plasticity (table 2, process 1; fig. A1A; West-Eberhard 1989, 2003).

A reduction of within-individual variance contributed to increased repeatability in gizzard mass in juveniles between year 1 and year 2. In year 1, the within-individual variance in gizzard mass was higher in juveniles than in adults. However, between year 1 and year 2, within-individual variance in juveniles decreased, while we found no year-related differences in within-individual variance in adults (table 4). Taken together, we interpret the decrease in within-individual variance found in juveniles as canalization of gizzard mass during ontogeny (table 2, process 3).

The absence of age-related differences in repeatability of exploration behavior concealed underlying changes in variance components in both juveniles and adults. Within-individual variance in exploration decreased significantly between year 1 and year 2 for both juveniles and adults. Concomitant (nonsignificant) decreases in among-individual variance meant that there was no overall change in repeatability of exploration between year 1 and year 2 (table 3). As we found no systematic change in average exploration behavior between year 1 and year 2 (table 3; fig. 2), we rule out the possibility that the decrease in within-individual variance in exploration from year 1 to year 2 (table 3) was the result of habituation to the experimental arena. Instead, the decrease in within-individual variance is consistent with the idea of canalization (table 2, process 3). Because this decrease in within-individual variance was quantitatively similar for juveniles and adults, we consider the possibility that this decrease in within-individual variation may reflect a “time in captivity” effect, as opposed to a strictly developmental process (that would result in changes in the juvenile cohort alone). Since we found no increase in among-individual variance in exploration behavior in either juveniles and adults, we suggest that among-individual differences in developmental plasticity in exploration were not at play during our experiments (table 2, process 1).

Taken together, our results suggest that canalization may play an important role in the development of among-individual differences for both gizzard mass and exploration. Importantly, our results also demonstrate that studying age-related differences in repeatability alone, without considering the differences in among-individual and within-individual

variance components separately, is insufficient for studying developmental processes. This is because no overall change in repeatability can occur even when there are significant changes in the underlying variance components if these exhibit changes in the same direction.

We acknowledge that these trait-specific processes may be partially caused by aspects of our experimental design. Notably, our diet manipulation did not have any effect on mean exploration, even in juveniles (fig. 2). This indicates that if there is a sensitive window during early development in which knots adjust their exploration behavior directly in response to changes in diet quality, it occurs earlier in development than the timescale of our experiments (i.e., 4 months of age). Consequently, we repeatedly induced within-individual shifts in gizzard mass but not in exploration. This may have allowed for greater reduction in within-individual variance in exploration in the course of the study compared with gizzard mass. As with exploration behavior, there was no evidence that juveniles were more sensitive to diet manipulations than adults in terms of adjustment in gizzard mass (table 4), because overall diet effects were similar for adults and juveniles within each study year.

By offering standardized aviary conditions, we deliberately separated the individual from its natural environment as a way of explicitly controlling for factors that might influence physiology and behavior (Gibbons et al. 1994). Contrary to free-living knots, the birds in the context of our experiments lived in absence of competition for food and actual predation danger; these are potentially important factors known to contribute to among-individual variation (e.g., Bengtson et al. 2014; Urszán et al. 2015; but see Horváth et al. 2017). The lower among-individual variance in gizzard mass and exploration in juveniles when compared to adults in year 1 indicates that among-individual variation increases over time in free-living knots. Our results therefore suggest that environmental conditions play a key role in shaping patterns of phenotypic (co)variation in knots.

In addition, whereas covariation between gizzard mass and exploration behavior has been reported in free-living knots (Bijleveld et al. 2014), we replicated the earlier finding that there is no covariation between the gizzard mass and exploration in captivity (Bijleveld et al. 2014). At the same time, among-individual variation in both gizzard mass and exploration behavior between year 1 and year 2 eroded in adults, and not in juveniles, suggesting that our captive conditions indeed removed a variable that generates and maintains among-individual variation (e.g., Archard and Braithwaite 2010; Fisher et al. 2015). However, our results are consistent with Bijleveld et al. (2014) and Oudman et al. (2016), who both found experimental manipulations of gizzard mass not to affect the expression of exploration behavior. Thus, repeatable variation in field gizzard mass may be considered pseudorepeatable (Niemelä and Dingemanse

2017; i.e., it is the outcome of repeatable exposure to different conditions such as habitat or diet). Indeed, the observed among-individual variance in gizzard mass was greater in two earlier studies where the choice for prey quality was greater (Bijleveld et al. 2014) and the time in captivity was shorter (Mathot et al. 2017).

At least two other studies have reported how within-individual canalization can contribute to increased repeatability. In sea anemones (*Actinia equina*), within-individual variation in the startle response decreased over time (Osborn and Briffa 2017). Similarly, a reduction of within-individual variance explained the strong age-related increase in repeatability in multiple behavioral traits in mosquitofish (*Gambusia holbrooki*; Polverino et al. 2016; table 1). We suggest that reduction in within-individual variation may be the outcome of Bayesian updating (Stamps and Krishnan 2017). With increased exposure to environmental cues, individuals may be more certain in their assessment of the environment. As their estimate becomes more accurate, smaller phenotypic adjustments are needed (Stamps and Krishnan 2014b).

Returning to Waddington's (1942) metaphor of canalization, it is likely that the exact canalization process, and the adaptive value of within-individual canalization, varies between traits and that some traits are shaped more rigidly than others. The limited level of canalization of gizzard mass found here may be explained by the fact that there is strong selection to retain plasticity in gizzard mass. Red knots benefit by being able to fine-tune gizzard mass to seasonally changing diets and highly variable food conditions at the nonbreeding grounds (Zwarts and Blomert 1992; Piersma et al. 1993; Battley and Piersma 2005). The strong within-individual canalization of exploration, on the other hand, might be the result of a few environmental switches during early development (Waddington 1942); it suggests that having a predictable expression of exploration behavior (that varies between individuals) is beneficial, even early in life. The individual exploration behavior in red knots may well start as the hatchling chicks begin to forage and explore their tundra birthplaces.

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