

Recurrent violations of invariant rules for offspring size: evidence from turtles and the implications for small clutch size models

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Abstract Smith and Fretwell's classic model predicts that parents can maximize fitness by dividing the energy available for reproduction into offspring of an optimal size. However, this model breaks down when clutch size is small (~ 1 –10 offspring). Invariant rules are an extension of the Smith–Fretwell model, and these rules predict how offspring size will vary among and within individuals that produce small clutch sizes. Here, we provide a narrow test of invariant rules using three turtle species, then we synthesize and re-analyze existing data from 18 different species (comprising five Orders) to evaluate whether invariant rules are followed across broad taxa. We do not find support for most invariant rules in turtles, and our

re-analysis demonstrates a general mismatch between observed and expected values across all taxa evaluated, suggesting that invariant rules fail to predict reproductive patterns in nature. Morphological constraints on offspring size and reproductive effort may be important reasons for disparities between theory and observation both in turtles and other taxa. Paradoxically, morphological constraints are most common in small-bodied species and individuals, but these same candidates are also those which produce the small clutch sizes that are necessary to test invariant rules, such that a fair test of invariant rules will often be challenging. Mismatches between theory and observation might also occur because theory assumes that mothers exert control over resource allocation to offspring. In fact, there is evidence of widespread genetic correlations among investment per offspring and reproductive effort, such that these traits are not independent.

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Introduction

A major goal of life-history research is to understand how reproductive schedules, age at maturity, and investment per offspring evolve in response to environmental variation and selection on other characters. Yet, assessing the adaptive significance of life-history traits can be difficult. For example, genetic and phenotypic correlations among characters can obscure the traits being targeted by selection (Lande and Arnold 1983), and a failure to correctly identify these targets can undermine our understanding of trait combinations that maximize fitness (Gilchrist and

Kingsolver 2001). Adaptive evolution of some characters may also be limited by morphological constraints, such that a major portion of observed phenotypic variation is a direct consequence of phylogeny and body morphology.

One method of disentangling adaptive variation from variation owing to genetic and morphological constraints is to estimate the theoretical value of the trait that confers maximum fitness to individuals, and then to test whether values observed in natural populations are similar (Orzack and Sober 1994; Abrams 2001). To estimate optimal phenotypes, one must assume that natural selection is the only important evolutionary force acting on the trait of interest (Orzack and Sober 1994), and that the expression of the trait is governed only by a particular set of underlying relationships with other characters. The resultant optimality model generates specific predictions that can then be tested against observed values. When empirical observations do not quantitatively match model predictions, the observer can examine the model and assess which fundamental principles or assumptions have been violated, and whether constraints exist (Abrams 2001). A more complete understanding of the relationships among correlated traits may result, so new assumptions can be incorporated into a subsequent model, and model accuracy can be re-assessed.

Perhaps the most influential optimality model was developed by Smith and Fretwell (1974). This classic model of offspring size evolution affirms that parental reproductive success is maximized by balancing the trade-off between offspring quantity and quality, and it predicts that a single level of investment per offspring (offspring size) will maximize parental reproductive success in a given environment. More recently, Charnov and Downhower (1995) recognized that a problem arises when applying this classic model to species that produce clutch or litter sizes of less than about 10 offspring. Given that the predicted clutch size (under an optimality model) is usually not an integer, a parent with resources intermediate to those required for the production of three or four eggs (for example) must produce either three eggs of greater than optimal size, or four eggs that are smaller than the optimal size (Ricklefs 1968; Ebert 1994). An interesting consequence of this problem is that the ratio of the ranges of offspring size in clutches of size i and $i + 1$ will be inversely proportional to the ratio of clutch sizes,

$$\frac{Imax_{i+1} - Imin_{i+1}}{Imax_i - Imin_i} = \frac{C_i}{C_{i+1}} \quad (1)$$

where C_i is a clutch (or litter) of size i , and I is offspring size such that $Imax_i$ and $Imin_i$ are the maximum and minimum offspring sizes observed in clutches of size i (Charnov and Downhower 1995). The most compelling aspect of this prediction (henceforth ‘Invariant Rule 1’) is that it appears to hold over a wide range of relationships

between offspring size and fitness, and it can be generalized to all species in which the following six assumptions hold: (1) resources are allocated equally to offspring within clutches; (2) optimal offspring size does not change across levels of clutch size; (3) conversion of resources into per offspring investment is linear (see Downhower and Charnov 1998); (4) there is effectively no lower or upper limit on offspring size; (5) mothers exhibit control over resource allocation; and (6) resources available for reproduction are fixed and represent total investment for a given reproductive episode. Assumptions 1–4 can be usually be verified by the researcher, and they should guide the choice of model organism (e.g., Uller and Olsson 2009), whereas assumptions 5 and 6 are more difficult to verify and relate more directly to fundamental assumptions of classic egg size theory (Smith and Fretwell 1974). These six assumptions, in conjunction with Invariant Rule 1, can be collectively referred to as Charnov and Downhower’s (1995) model. When properly applied, this model is a powerful tool for testing fundamental tenets of egg size theory and, ultimately, for ameliorating our understanding of reproductive variation in the wild.

Large datasets are necessary to test Invariant Rule 1, and, to date, only a handful of studies have examined its prediction (Charnov et al. 1995; Guinnee et al. 2004, 2005; Kasparian et al. 2005; Uller et al. 2009; Uller and Olsson 2009; West et al. 2001). Yet, even within the restricted range of taxa examined, support for the rule is equivocal. On the one hand, a common finding is that the range of offspring sizes decrease with increasing clutch size, which is interpreted as qualitative support for Invariant Rule 1 (Guinnee et al. 2005). On the other hand, quantitative matches between observed and predicted values are usually associated with large error estimates (i.e., large confidence intervals), which is interpreted as weak support for the rule (Uller et al. 2009). However, many studies have not verified all testable assumptions of the model or focus on species that are unlikely to conform to these assumptions (Kasparian et al. 2005; Uller et al. 2009), and it is therefore difficult to assess the current level of support for Invariant Rule 1. A clear discussion of how violating the underlying model assumptions can cause deviations from predicted values is warranted.

The first objective of the present study is to test Charnov and Downhower’s (1995) model using reproductive data from three species of freshwater turtles (snapping turtles, *Chelydra serpentina*; wood turtles, *Glyptemys insculpta*; and painted turtles, *Chrysemys picta*). Given that egg size and reproductive effort are constrained to varying degree in these three species (Congdon and Gibbons 1987; Rollinson and Brooks 2007; Rollinson et al. 2012), the second objective is to clarify how morphological constraints on offspring provisioning can cause disparities between

observed values and those predicted under Charnov and Downhower's model. Lastly, by synthesizing all existing tests of Invariant Rule 1, we evaluate how and under what conditions Charnov and Downhower's model has failed to explain patterns of reproductive variation, and we examine the extent to which morphological constraints may have played a role in previous failures of the model.

Materials and methods

The present study uses four different datasets. One dataset exists for each of our three turtle species, and these data are used to test Invariant Rule 1 and Assumptions 1–4 for each species. A fourth dataset was compiled from the literature and incorporates every known estimate of the left-hand side of Eq. 1 for a variety of taxa (i.e., tests of Invariant Rule 1). This dataset is used to test whether Invariant Rule 1 is broadly supported across taxa.

Species descriptions and data collection

Painted turtles are small-bodied turtles in the family Emydidae. Mature females typically weigh 500 g and can reach approximately 800 g in mass. Painted turtles lay 1–14 eggs per clutch with up to two clutches per year. Wood turtles are larger-bodied members of Emydidae. Mature females typically weigh 1,200 g and they can reach approximately 1,500 g. Wood turtles lay a single clutch of 3–15 eggs each year. Snapping turtles are a much larger species of turtle and they belong to the family Chelydridae. Mature females typically weigh 5,000 g and they can reach 9,500 g. Snapping turtles lay one clutch per year comprising 20–60 eggs. Small clutch size models should not, therefore, apply to snapping turtles, but this species was included to provide insight into how constraints on offspring provisioning affect tests of invariant rules.

Clutch data from wild populations of painted turtles, wood turtles and snapping turtles were collected as part of an ongoing study in Algonquin Provincial Park, Ontario, Canada. Data were collected from nest sites in close proximity to one another and are considered to represent a single population of each species (for details, see Brooks et al. 1991; Rollinson et al. 2008, 2012). Sampling years vary among species prior to 1990, but are generally continuous for all species from 1990 to 2012, although wood turtles were monitored less intensively. For each species, every clutch laid was excavated, and individual eggs were counted and weighed to the nearest 0.1 g. In these populations, all females are uniquely identified by an alphanumeric code, which allowed each clutch to be attributed to an individual female. A subset of painted turtles will lay

two clutches each year, and given that mean egg size is smaller in the second clutch (Rollinson and Brooks 2008b), it is conceivable that optimal offspring size differs between clutches. To ensure data only from first clutches were used (i.e., one optimal offspring size), we excluded data from clutches that were laid after the first 15 days of nesting season onset. Following Charnov et al. (1995), levels of clutch size were excluded from our analysis if there were fewer than 10 clutches, such that n was always ≥ 10 when estimating the variance in mean egg mass among clutches of a given size.

Analysis of turtle data

The first stage of our analyses examined three testable assumptions of the Charnov and Downhower (1995) model for each species using the species-specific datasets described above. First, we tested the assumption that resources are allocated equally to eggs within a clutch (Assumption 1) with a randomization procedure. For each level of clutch size, we compared the variance in egg mass observed within clutches to the variance in egg mass expected if clutches were composed of eggs selected at random from any clutch of the same size. We used a bootstrapping procedure to produce 10,000 randomized clutches for each clutch size and calculated the variance in egg mass within each randomized clutch. We compared the 5th percentile of this distribution to the observed distribution of within clutch egg mass variation.

Next, we indirectly examined the assumption that there is a single optimal egg size (Assumption 2). For each species, egg mass data were averaged within individual clutches to produce one mean estimate of egg mass per clutch, then mean egg mass for each clutch size was calculated. Assumption 2 was evaluated by testing for a correlation between mean egg mass and clutch size for each species using a Spearman's rank correlation. We deemed Assumption 2 to be upheld if there was no statistically significant relationship between clutch size and mean egg mass.

The final assumption we tested is that the conversion of resources into per offspring investment is linear (Assumption 3), or that the increase in energy devoted to reproduction that is required to go from a clutch of size i to a clutch of size $i + 1$ does not depend on clutch size (Downhower and Charnov 1998). For each species, we modeled linear and quadratic relationships between clutch mass and clutch size, with the expectation that the data would best support a linear relationship between clutch mass and clutch size. Clutch mass was estimated as the product of clutch size and mean egg mass of individual clutches. The linear model was,

$$Clutch\ Mass_{kl} \sim \beta_0 + \beta_1 \cdot Clutch\ Size_{kl} + \gamma_l + \varepsilon_{kl} \quad (2)$$

Where *Clutch Mass* is the total mass of the clutch *k*, *Clutch Size* is the number of eggs in clutch *k*, γ is the random intercept for female *l*, β are the parameters to be estimated, and ε is error. The second model for each species featured a quadratic term for clutch size,

$$Clutch\ Mass_{kl} \sim \beta_0 + \beta_1 \cdot Clutch\ Size_{kl} + \beta_2 \cdot Clutch\ size_{kl}^2 + \gamma_l + \varepsilon_{kl} \quad (3)$$

Both models used maximum likelihood parameter estimation, and we followed an information-theoretic approach to compare models using AICc (Burnham and Anderson 2002). All statistics were performed using R (R Development Core Team 2011), using the packages lme4 (Bates and Maechler 2010) and AICcmodavg (Mazerolle 2010).

The second stage of our analyses tested one qualitative and one quantitative prediction of Charnov and Downhower's model. Egg mass data were averaged within individual clutches, such that one estimate of mean egg mass existed for every clutch in our dataset. For each turtle species, we tested the qualitative prediction that the variance in mean egg mass observed among individuals that produce a particular clutch size will decrease as clutch size increases. These tests were performed using an ordered heterogeneity test (Rice and Gaines 1994), which is a non-directional test that combines the Spearman's rank correlation (r_s) with the complement of the *P* value from a Bartlett's test of homogeneity of variance (P_c). Next, these same datasets were used to test Invariant Rule 1, which affirms quantitative agreement between the right-hand and left-hand sides of Eq. 1. We calculated the ratio of the ranges of mean egg mass (i.e., estimates of the left-hand side of Eq. 1) for clutch sizes $\{i+1, i\}$, and tested whether the theoretical prediction (given by the right-hand side of Eq. 1) was within the 95 % confidence interval of our estimate. Confidence intervals were calculated by conducting 10,000 bootstrapped simulations for each of the between clutch size comparisons. In these simulations, the smallest sample size for each clutch size comparison was used.

Literature synthesis and re-analysis

We surveyed the literature and synthesized all studies that estimated the ratio of the ranges of offspring size for clutch sizes $\{i+1, i\}$ between $\{2,1\}$ and $\{13,12\}$ and compared these estimates to the theoretical prediction of C_i/C_{i+1} . In total, this dataset consisted of 113 ratios estimated from 18 different species comprising 10 different families (Online Resource 3). A mean-parameterized mixed analysis with restricted maximum likelihood parameter estimation was

used to model these estimates, using the package lme4 (Bates and Maechler 2010). Family was modeled as a random intercept ($n = 10$ Families, 18 Species) to control for any differences in mean values observed among families. We also included a random intercept that controls for mean differences among populations that originally produced the estimates. Clutch sizes $\{i+1, i\}$ from which the ratio of the ranges of offspring size were estimated were modeled as fixed effects for $\{2,1\}$ through $\{13,12\}$,

$$Observation_{km:l} \sim \beta_1 \cdot C\{2,1\}_{km:l} + \beta_2 \cdot C\{3,2\}_{km:l} + \dots + \beta_{12} \cdot C\{13,12\}_{km:l} + \gamma_l + \delta_{m:l} + \varepsilon_{km:l} \quad (4)$$

where *Observation* is the estimate *k* of the ratio of the ranges of offspring size, $C\{2,1\}$ through $C\{13,12\}$ are the fixed effects of clutch sizes $\{i+1, i\}$, γ is the random intercept of family *l*, and δ is the random intercept of population *m*, nested within family. The parameters to be estimated are β , and ε is error. Highest posterior density intervals (i.e., 95 % credible intervals) were estimated from the posterior distribution of the parameters of the fitted mixed model using the functions HPDinterval and mcmcSamp within the lme4 package ($n = 5,000$ samples).

Results

Turtle Data

We analyzed 2,034 painted turtle clutches ranging in size from 3 to 12 eggs, 199 wood turtle clutches ranging from 6 to 13 eggs, and 1,133 snapping turtle clutches ranging from 23 to 51 eggs (Table 1). Across all species, Assumption 1 was generally satisfied: eggs within a clutch were of similar size. The observed within-clutch variance was less than that expected by chance in 7 of 10 comparisons for painted turtles, 7 of 8 comparisons for wood turtles, and in 30 of 30 comparisons for snapping turtles (Online Resource 1). Conversely, the assumption that mean egg mass does not change across levels of clutch size (Assumption 2) was not satisfied in two of our three species. There was a negative relationship between mean egg mass and clutch size in painted turtles (Spearman's $\rho = -0.891$, $n = 10$, $P = 0.001$), and this relationship was positive in snapping turtles (Spearman's $\rho = 0.497$, $n = 27$, $P = 0.006$). However, Assumption 2 was satisfied in wood turtles, as there was no correlation between mean egg size and clutch size (Spearman's $\rho = 0.333$, $n = 8$, $P = 0.43$; Fig. 1).

The assumption that conversion of resources into eggs is linear (Assumption 3) was satisfied in only one species of turtle. For painted turtles ($n = 336$ females; 2,043 clutches) and snapping turtles ($n = 268$ females; 1,214 clutches), substantial support for models describing a quadratic relationship between clutch mass and clutch size was

Table 1 Tests of Invariant Rule 1

Species	Clutch sizes (C_i, C_{i+1})	No. clutches	Expected (C_i/C_{i+1})	Observed	Lower CI (2.5 %)	Upper CI (97.5 %)
Painted turtle	(3, 4)	(19, 56)	0.750	1.10	0.605	1.60
	(4, 5)	(56, 157)	0.800	0.922	0.644	1.20
	(5, 6)	(157, 364)	0.833	0.934	0.743	1.13
	(6, 7)	(364, 549)	0.857	1.19	0.990	1.38
	(7, 8)	(549, 465)	0.875	0.775	0.672	0.878
	(8, 9)	(465, 253)	0.889	1.03	0.893	1.16
	(9, 10)	(253, 125)	0.900	0.815	0.659	0.971
	(10, 11)	(125, 33)	0.909	1.21	0.808	1.60
Wood turtle	(11, 12)	(19, 56)	0.917	0.750	0.265	1.24
	(6, 7)	(10, 13)	0.857	1.06	0.246	1.88
	(7, 8)	(13, 24)	0.875	1.180	0.434	1.93
	(8, 9)	(24, 32)	0.889	0.776	0.414	1.14
	(9, 10)	(32, 42)	0.900	1.04	0.644	1.44
	(10, 11)	(42, 35)	0.909	1.03	0.581	1.47
Snapping turtle	(11, 12)	(35, 29)	0.917	0.868	0.358	1.38
	(12, 13)	(29, 14)	0.923	0.917	0.347	1.49
	(23, 24)	(16, 10)	0.958	0.986	0.460	1.51
	(24, 25)	(10, 12)	0.96	1.29	0.0466	2.53
	(25, 26)	(12, 28)	0.962	0.816	0.361	1.27
	(26, 27)	(28, 35)	0.963	1.11	0.652	1.58
	(27, 28)	(35, 37)	0.964	0.796	0.531	1.06
	(28, 29)	(37, 40)	0.966	1.10	0.900	1.29
	(29, 30)	(40, 45)	0.967	1.28	0.827	1.73
	(30, 31)	(45, 52)	0.968	0.788	0.728	0.849
	(31, 32)	(52, 63)	0.969	0.856	0.662	1.05
	(32, 33)	(63, 57)	0.970	1.66	1.20	2.12
	(33, 34)	(57, 70)	0.971	0.819	0.522	1.12
	(34, 35)	(70, 68)	0.971	0.778	0.556	1.00
	(35, 36)	(68, 67)	0.972	1.18	1.01	1.35
	(36, 37)	(67, 64)	0.973	1.00	0.805	1.20
	(37, 38)	(64, 51)	0.974	0.885	0.648	1.12
	(38, 39)	(51, 64)	0.974	1.31	0.875	1.74
	(39, 40)	(64, 40)	0.975	1.167	0.623	1.71
	(40, 41)	(40, 43)	0.976	0.805	0.469	1.14
	(41, 42)	(43, 42)	0.976	1.01	0.560	1.46
	(42, 43)	(42, 46)	0.977	0.841	0.458	1.22
	(43, 44)	(46, 35)	0.977	1.113	0.674	1.55
	(44, 45)	(35, 25)	0.978	0.805	0.480	1.13
	(45, 46)	(25, 21)	0.978	0.539	0.241	0.836
	(46, 47)	(21, 20)	0.979	3.20	1.53	4.87
	(47, 48)	(20, 30)	0.979	0.550	0.265	0.835
	(48, 49)	(30, 16)	0.980	1.07	0.574	1.56
	(49, 50)	(16, 19)	0.980	0.842	0.230	1.46
	(50, 51)	(19, 17)	0.980	1.40	0.341	2.47

Observed is the quotient of the range in egg mass in clutches of size $i + 1$ divided by the range in egg mass in clutches of size i (see Eq. 1); upper and lower confidence intervals are for observed ratios

observed (Table 2). In both cases, quadratic terms were negative indicating a concave relationship, and the amount of energy devoted to reproduction at a given clutch size was lower than expected when clutch size was large (Fig. 2). In wood turtles ($n = 50$ females; 208 clutches),

AICc values were similar for the quadratic and linear models (Table 2), but model fit (as measured by log likelihood) was essentially the same, so the quadratic model was not supported (see Burnham and Anderson 2002: 131). Therefore, Assumption 3 was only supported for wood

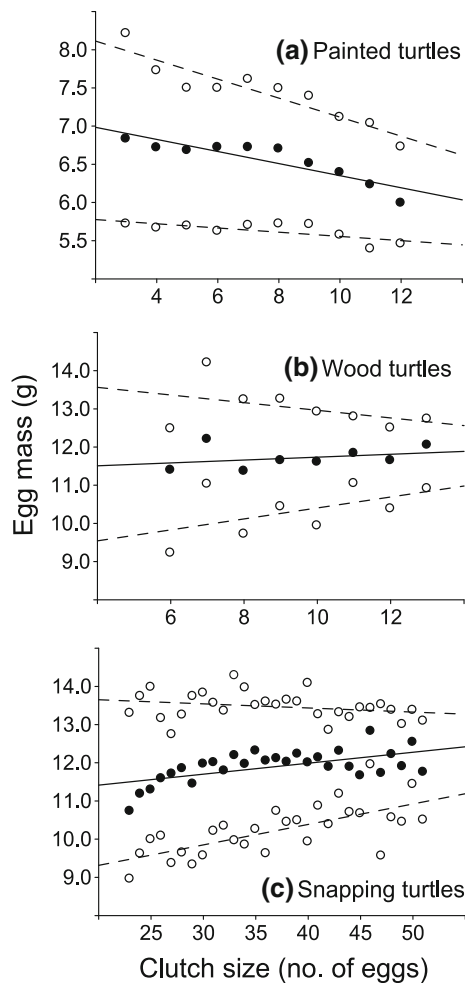


Fig. 1 Mean egg mass (closed circles) and its 10th and 90th percentiles (open circles) as a function of clutch size for three species of turtle: **a** painted turtles (*Chrysemys picta*), **b** wood turtles (*Glyptemys insculpta*), and **c** snapping turtles (*Chelydra serpentina*). Dashed lines are linear trend lines for 10th and 90th percentiles as a function of clutch size, and the solid line is the linear trend line for mean egg mass

turtles, although the sample size was low (see Online Resource 2 for model summaries).

The qualitative prediction that variation in egg mass should decrease with increasing clutch size was upheld in all turtle species. Ordered heterogeneity tests indicated that there was a negative correlation between clutch size and egg mass variation for painted turtles ($P_c = 0.999$, $r_s = -0.915$, $rsP_c = -0.914$, $P < 0.001$), wood turtles ($P_c = 0.912$, $r_s = -0.929$, $rsP_c = -0.852$, $P < 0.001$), and surprisingly, for snapping turtles ($P_c = 0.999$, $r_s = -0.657$, $rsP_c = -0.657$, $P < 0.001$). However, the observed 10th and 90th percentiles of egg mass variation did not converge symmetrically on the mean egg mass in two species, nor was the nature of the asymmetric convergence consistent among species. In painted turtles, egg mass decreased across levels of clutch size, but minimum

Table 2 Relative support for linear and quadratic mixed-effect models describing relationships between clutch mass and clutch size for each turtle species (see Eqs. 2 and 3)

Species	Model	K	AICc	$\Delta AICc$	Log likelihood
Painted turtle	Linear	4	11,473.8	29.2	-5,732.9
	Quadratic	5	11,444.6	0	-5,717.3
Snapping turtle	Linear	4	12,286.8	12.1	-6,139.4
	Quadratic	5	12,274.7	0	-6,132.3
Wood turtle	Linear	4	1,534.4	0	-763.1
	Quadratic	5	1,535.6	1.2	-762.7

The number of parameters in the model is K . See Online Resource 2 for summary of best predictive models

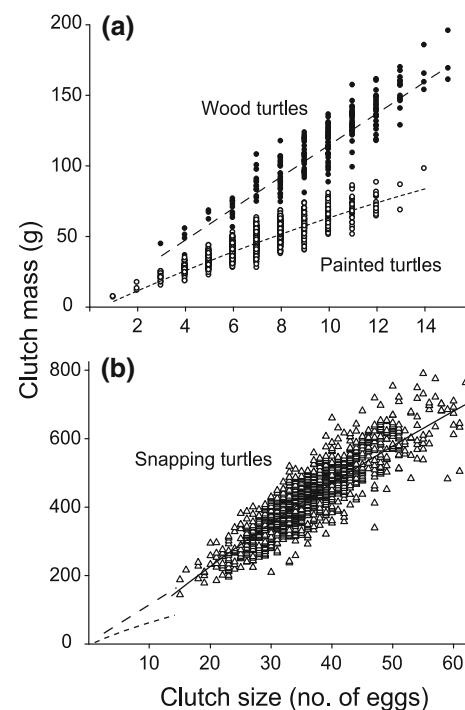


Fig. 2 Relationships between clutch mass and clutch size in **a** painted turtles (open circles, dotted line) and wood turtles (closed circles, dashed line), and **b** snapping turtles (triangles, solid line) estimated from mixed-effect models (Table 2; Online Resource 2). Trend lines for painted turtles (dotted line) and wood turtles (dashed line) are shown alongside snapping turtle data for comparison. Sample sizes were 336 females and 2,043 clutches for painted turtles, 50 females and 208 clutches for wood turtles, and 268 females and 1,214 clutches for snapping turtles

egg mass remained relatively constant and maximum egg mass decreased substantially (Fig. 1a). The opposite pattern was observed in snapping turtles, where maximum egg size was relatively constant, but mean and minimum egg size increased across levels of clutch size (Fig. 1c). Testing this qualitative prediction of the Charnov and Downhower model therefore revealed a violation of Assumption 4 in two species: we observed an upper limit on egg size in snapping turtles, and a lower limit on egg size in painted

turtles. Convergence was symmetrical for wood turtles (Fig. 1b), and there was no evidence of limits on egg size.

For all turtle species, we observed quantitative support for Invariant Rule 1, as there was general agreement between observed and expected values (painted turtles, 7 of 9 comparisons; wood turtles, 7 of 7 comparisons; snapping turtles, 28 of 28 comparisons; Table 1). However, 95 % confidence intervals overlapped 1.0 in most cases, indicating that there was effectively no change in the ratio of the ranges of egg size from one level of clutch size to the next.

Literature synthesis and re-analysis

Our re-analysis of data synthesized from the literature revealed a striking pattern: all estimates of the ratio of the ranges of offspring size for clutch sizes {2, 1} fell above the predicted value of 0.50 (see Online Resource 3 for raw data). A similar trend was apparent at clutch sizes {3, 2} and {4, 3} where the vast majority of estimates fell above the predicted value (Fig. 3a). Highest posterior density intervals suggest observed values are greater than predicted values when clutch sizes are low (Fig. 3b), but confidence intervals become larger and overlap predicted values as clutch sizes become larger. Contrary to the prediction of Invariant Rule 1, the ratio of the ranges of offspring size did not decrease with increasing clutch sizes across broad taxa; in fact, the observed ratio was centered on 1.0 for all clutch sizes (except for clutch sizes {5, 4}). The phylogenetic signal (the effect of family) was weak, suggesting that for a given taxon the average observed value fit no better or worse to predicted values (see Online Resource 4 for model summary). However, data were not sufficient to model whether the ratio of the ranges of offspring size decreased relatively slowly or quickly for particular taxonomic groups, such that we could not perform a detailed examination of how phylogeny was related to deviations from predicted values. Notwithstanding, both the qualitative analysis (Fig. 3a) and quantitative analysis (Fig. 3b) suggest that variation in offspring size does not decrease following Eq. 1 when clutch sizes are smallest.

Discussion

In the present study, we tested assumptions and predictions of the Charnov and Downhower model in three species of turtles where egg size and reproductive effort are constrained to varying degrees. We also tested whether the main prediction of Charnov and Downhower's model (Invariant Rule 1) is upheld across broad taxa by synthesizing and re-analyzing all data existing in the literature. We found that turtles violated many assumptions and predictions of the model and Invariant Rule 1 was not

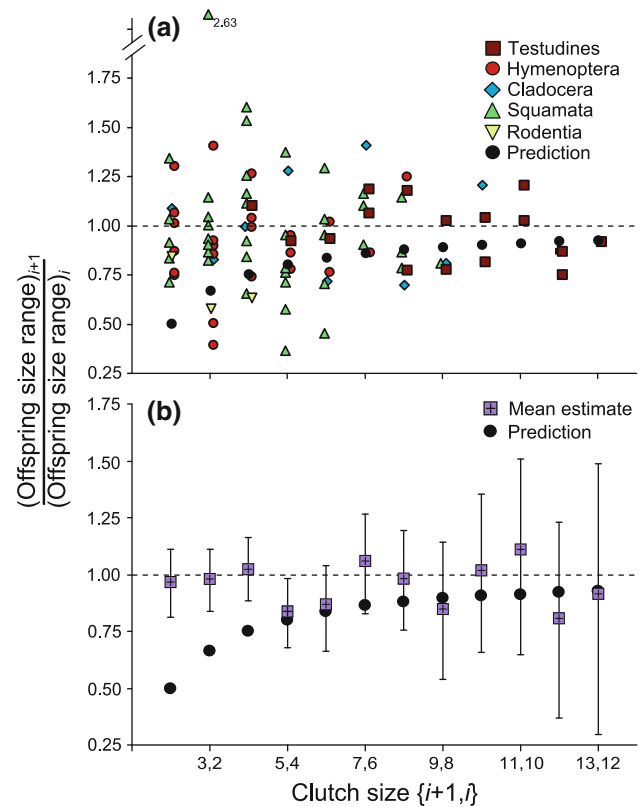


Fig. 3 **a** To examine whether Invariant Rule 1 is broadly supported, all known estimates of the ratio of the ranges of offspring size (i.e., the left-hand side of Eq. 1) for clutch sizes $\{i+1, i\}$ for five different taxonomic orders were plotted alongside predicted values (i.e., the right-hand side of Eq. 1). The dashed line is where the ratio of the ranges of offspring size for clutch sizes $\{i+1, i\}$ equals 1.0, which indicates that there is effectively no change in the range of offspring size from one level of clutch size to the next. For clarity, some estimates are offset from the x-axis, and estimates for different sexes of the same species are averaged within levels of clutch size. See Online Resource 3 for raw data. **b** Mean values (± 95 % highest posterior density intervals) estimated for the left-hand side of Eq. 1 from a mixed-model ANOVA are plotted alongside predicted values (see Online Resource 4 for a summary of the mixed model). To satisfy Invariant Rule 1, confidence intervals should encompass the predicted value, but they should not encompass the dashed line

upheld across broad taxa. However, patterns of egg size variation differed among all three turtle species, and when viewed together, these patterns help explain why our re-analysis revealed widespread deviations from model predictions. Below, we illustrate how a lack of agreement between observed and expected values in turtles and other taxa can be attributed to constraints on minimum and maximum egg size, to morphological constraints on clutch volume and reproductive effort, and to genetic correlations between investment per offspring and other traits such as reproductive effort.

An assumption of Charnov and Downhower's model is that there is effectively no upper or lower limit to egg size. However, in reality, there must be a minimum level of

investment per offspring that is necessary for offspring to be viable (Smith and Fretwell 1974; Rollinson and Hutchings 2013). In painted turtles, minimum egg size produced at a given level of clutch size was constant, but both the mean and maximum egg size decreased as clutch size increased, causing asymmetric convergence of egg size variation on mean egg size (Fig. 1a). This pattern ultimately led to a non-linear conversion of resources into clutch size (Fig. 2a), and it is probably due to an encroachment of minimum egg size on minimum viable offspring size (Guinnee et al. 2004; Kratochvil and Frynta 2006). Painted turtles are indeed the smallest-bodied species evaluated here, and they also produce the smallest eggs of our three species, so it is not surprising that egg size is relatively prone to encroachment on minimum viable size.

Although minimum egg mass was constant in painted turtles, mean and maximum egg mass decreased with increasing clutch size (Fig. 1). A decrease in mean egg mass across levels of clutch size has generally been interpreted as evidence that optimal offspring size is different for different clutch sizes (e.g., Guinnee et al. 2004; Uller et al. 2009; Uller and Olsson 2009). However, a more parsimonious explanation for this correlation arises from simple geometric principles. When the body cavity or brood pouch is relatively small, volumetric constraints stipulate that, in general, increases in clutch size must be strongly associated reductions in egg size (Glazier 2000). On the other hand, larger body cavities can accommodate more eggs without any necessary reduction in egg size. Therefore, for volumetric reasons, mean egg size of small-bodied individuals will decrease relatively quickly with increases in clutch size, even though an energetic trade-off between investment per offspring and fecundity occurs both in small-bodied and large-bodied individuals (Glazier 2000). This principle (Glazier's principle) can explain why mean and maximum egg mass decreased with clutch size in small-bodied painted turtles, and why mean egg mass did not decrease with clutch size in larger-bodied wood turtles and snapping turtles (Fig. 1).

Snapping turtles, the largest of our three species, produce clutch sizes that are between 20 and 60 eggs, but the Charnov and Downhower (1995) model is expected to apply only to clutch sizes that are less than about 10 eggs. Nonetheless, we still observed a negative correlation between egg size variation and clutch size, a qualitative pattern predicted by Charnov and Downhower's model. Interestingly, the pattern of asymmetric convergence was opposite to that observed in painted turtles (Fig. 1c): mean and minimum egg size increased as clutch size increased, and maximum egg size was constant across levels of clutch size. This suggests a constraint on maximum egg size of snapping turtles. One possibility is that optimal egg size is relatively small in this population, such that natural

selection has limited the evolution of greater egg size (Ji et al. 2006). More likely, however, is that maximum egg size is restricted by size of the pelvic aperture in snapping turtles (Congdon and Gibbons 1987; Ebert 1994). For instance, snapping turtles in the present study produced relatively small eggs only over a very narrow range of clutch sizes (where clutch was ~23–30 eggs; Fig. 1c), and the mean ($\pm$ SE) body size of snapping turtles producing these small clutches ($4,620 \pm 63.1$ g, $n = 182$) was much smaller than that of mothers producing clutches of more than 30 eggs ($5,570 \pm 35.1$ g, $n = 764$). This is consistent with the classic pattern of aperture-size constraints on maximum egg size (Congdon and Gibbons 1987; Rollinson and Brooks 2008a; also see fig. 2c in Ebert 1994). Notably, the fact that egg size increased with clutch size in snapping turtles ultimately resulted in non-linear conversion of resources into clutch size (Fig. 2b), such that different types of reproductive constraints in painted turtles (a body volume constraint) and snapping turtles (a pelvic aperture constraint) led to the same violation of Assumption 3.

No previously studied species supports all invariant rules, either because one or more assumptions were violated or because not all invariant rules were tested (Online Resource 5). Wood turtles, a species of intermediate body size, conformed to predictions of Charnov and Downhower's (1995) model and to the extensions derived by Downhower and Charnov (1998), making it the first example of a species in which all invariant rules were supported. However, we note that confidence intervals estimated for the left-hand side of Eq. 1 always overlapped 1.0 (Table 2), and that symmetric convergence of egg size variation on mean egg size was largely driven by a peculiar data point at clutch size seven (Fig. 1b), which has a sample size of only 13 clutches (Table 1). Removing this data point reveals a pattern identical to snapping turtles, such that more data are needed before concluding that wood turtles are the first example of universal support for this small clutch size model.

More broadly, we found that the main prediction of Charnov and Downhower's (1995) model (Invariant Rule 1) was not supported across taxa (Fig. 3), but we suspect that the lessons learned from studying invariant rules in turtles can provide insight into this general lack of support. Even though turtles have provided only a few narrow examples of how constraints on clutch volume, reproductive effort and size of the pelvic aperture can result in mismatches between theory and observation, we emphasize that species subject to similar morphological constraints on egg size and reproductive effort have been examined extensively in an invariant framework. For example, 13 of the 21 species for which invariant rules have been tested (not including turtles) comprise lizards and cladocerans (Online Resource 5), but morphological constraints on egg size and reproductive effort have been demonstrated in

both of these groups (Sinervo and Licht 1991; Boersma 1995). This taxonomic bias may have exacerbated the general lack of agreement between empirical observations and invariant predictions. Moreover, in 18 of the 24 species evaluated to date (Online Resource 5), mean egg size decreased with clutch size, and our painted turtle data suggest that constraints on clutch volume in small-bodied individuals and species may underlie this pattern (i.e., Glazier's principle). Ultimately, the present study points to a paradox for those wishing to test invariant rules: small-bodied species, or smaller individuals within a population, tend to produce the small clutch sizes required for testing invariant rules, but these same candidates are also prone to morphological constraints on egg size and reproductive effort which make them poor candidates for such tests (Sinervo and Licht 1991; Glazier 2000). If one is primarily interested in testing the accuracy of invariant rules, then this paradox underlines the need to consider the model organism very carefully.

On the other hand, many tests of invariant rules have been performed using parasitic wasps as model organisms (West et al. 2001; Guinnee et al. 2005), and morphological constraints on egg size probably do not affect offspring provisioning in most insects (e.g., Carriere et al. 1997; Bauerfeind and Fischer 2008). Widespread differences between observed and expected values in insects may also occur because maternal body size constrains offspring size in a different way. Genetic correlations among female size, growth, and follicular development (e.g., Czesak and Fox 2003; Bauerfeind and Fischer 2007) may restrict the extent to which investment per offspring and reproductive effort can evolve independently (Caley et al. 2001; Beck and Beck 2005). This undermines the assumption that females exert control over reproductive allocation (Assumption 5), which is a fundamental assumption of classic egg size theory (Smith and Fretwell 1974; Uller et al. 2009). Moreover, if reproductive effort and offspring size are generally not independent, then the range in offspring size observed at a given level of clutch size may be restricted. In this case, egg size variation would decrease with clutch size more slowly than predicted by Invariant Rule 1, which matches the results of our re-analysis.

Although there are reasons to suspect that empirical failure of invariant rules is partly an artifact of morphological constraints and partly indicative of genetic constraints, an added complication is that many studies have pooled egg size and clutch size data from mothers inhabiting different environments prior to performing tests of invariant rules (e.g., Charnov et al. 1995; Mayhew 1998; Guinnee et al. 2004; Uller et al. 2009). A fundamental prediction of classic theory is that optimal size is expected to vary among environments (Smith and Fretwell 1974). Therefore, if egg size differs among populations for

adaptive reasons, using data from multiple populations can inflate the variance in egg size at some or all levels of clutch size, which could lead to a conclusion that observed variation in egg mass does not decrease as quickly as Invariant Rule 1 predicts. This again matches the results of our re-analysis, and perhaps a closer agreement between observed and predicted values would occur if egg mass and clutch size data were not pooled across populations (e.g., Uller et al. 2009) or environments (e.g., Guinnee et al. 2004).

The present study draws from a narrow test of invariant rules using turtles to emphasize how morphological constraints on investment per offspring, such as pelvic aperture constraints and Glazier's principle, can cause a lack of agreement between observed and predicted values in an invariant framework. We have also provided three reasons why Invariant Rule 1 is not broadly supported. First, small-bodied species or individuals are those that tend to produce small clutch sizes, but these candidates are also most likely to experience morphological constraints on egg size and reproductive effort, making them unsuitable for testing invariant rules. For this reason, there are likely very few species that will conform to the assumptions of Charnov and Downhower's (1995) model. Second, pooling data from multiple populations has confounded several previous tests of invariant rules, and in these cases, disparities between observed and predicted values are not necessarily meaningful. Third, an unrealistic assumption underlies the Smith–Fretwell model in which invariant rules were founded: investment per offspring and reproductive effort are assumed to comprise independent processes, but several lines of evidence suggest that these traits are genetically correlated in many taxa, such that they cannot evolve independently. It has long been argued that the Smith–Fretwell model is too simplistic to describe the patterns observed in nature, and that more realistic assumptions are necessary if any size–number model is expected to generate accurate predictions (Bernardo 1996; Uller et al. 2009). We agree that some assumptions of classic theory are too simplistic, but we believe that testing invariant rules has contributed to a better understanding of offspring size variation in the wild (e.g., Guinnee et al. 2004; Kasparian et al. 2005) and has generated lucid, meaningful discussion about the assumptions that underlie classic theory (Smith and Fretwell 1974; Uller et al. 2009).

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