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Life history evolution in cichlids 2: directional evolution of the trade-off between egg number and egg size

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Abstract

The negative relationship between offspring number and offspring size provides a classic example of the role of trade-offs in life history theory. However, the evolutionary transitions in egg size and clutch size that have produced this negative relationship are still largely unknown. Since body size may affect both of these traits, it would be helpful to understand how evolutionary changes in body size may have facilitated or constrained shifts in clutch and egg size. By using comparative methods with a database of life histories and a phylogeny of 222 genera of cichlid fishes, we investigated the order of evolutionary transitions in these traits in relation to each other. We found that the ancestral large-bodied cichlids first increased egg size, followed by a decrease in both body size and clutch size resulting in the common current combination of a small-bodied cichlid with a small clutch of large eggs. Furthermore, lineages that deviated from the negative relationship between clutch and egg size underwent different transitions in these traits according to their body size (large bodied genera have moved towards the large clutch/small egg end of the continuum and small bodied genera towards the small clutch/large egg end of the continuum) to reach the negative relationship between clutch size and egg size. Our results show that body size is highly important in shaping the negative relationship between clutch size and egg size.

Introduction

The trade-off between clutch size and egg size is a common feature of both animals and plants (e.g. reviews by Roff, 1992; Stearns, 1992; Roff, 2002). The theory behind this trade-off is based on the assumption that the amount of material available to put into offspring is limited. Hence, females are selected to optimize rates of gain from investment into individual offspring against costs to the total number of offspring produced (e.g. Smith & Fretwell, 1974; Parker & Begon, 1986; Lloyd, 1987).

Empirical evidence for this trade-off has generally been based upon negative correlations among various taxa between the two traits (e.g. Roff, 1992, 2002; Stearns, 1992). For example, in teleost fishes, the trade-off between clutch size and egg size has received substantial empirical attention and is well described across species (see reviews by Elgar, 1990; Roff, 1992; Stearns, 1992; Einum *et al.*, 2004; Kolm *et al.*, 2005) as well as within species (e.g. Blaxter, 1969; Tanasichuk & Ware, 1987; Fleming & Gross, 1990; Snyder, 1990). Although a negative correlation cannot be taken as definitive evidence for a trade-off, this interpretation has been supported by experimental manipulations as well as demonstrations of a negative genetic correlation between the two traits (Snyder, 1991; Sinervo & Doughty, 1996; Schwarzkopf *et al.*, 1999). Moreover, comparative methods controlling for shared ancestry have been applied to a few taxa such as copepods (Poulin, 1995) and insects (Hymenopterans: Blackburn, 1991; Butterflies: Garcia-Barros, 2000), and they provide a more robust confirmation than simple correlation analyses based on species data that do not consider shared ancestry (Felsenstein, 1985; Harvey & Pagel, 1991). Therefore, it is widely

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accepted that the suggested trade-off between clutch size and egg size does indeed exist and is not simply an artefact from a third correlated factor (Roff, 2002).

Recent developments in comparative methods now allow for more detailed investigations of how traits have evolved in relation to each other. More specifically, the maximum likelihood method developed by Pagel (1994) has recently been used to infer how two traits may evolve dependent on each other. Such analyses have examined the temporal order of transitions between a variety of ecological and reproductive traits (e.g. Rolland *et al.*, 1998; Cézilly *et al.*, 2000; Krüger & Davies, 2002). We can now use these methods to investigate, in much more detail, how clutch size and egg size have evolved in relation to each other under the influence of a trade-off between the two.

In this study, we use cichlid fishes (*Cichlidae*), as a model system for examining the temporal sequence of transitions that have shaped the modern-day distribution of clutch and egg size relationships. Cichlid fishes are ideal for the study of these questions because of the large variation in all of these life-history traits, as well as in other potentially important ecological conditions within the family (e.g. Barlow, 1991,2000; Keenleyside, 1991; Goodwin *et al.*, 1998). Furthermore, there is a strong negative relationship between clutch size and egg size in cichlids also after accounting for phylogeny and body size (Kolm *et al.*, 2005). In light of the negative relationship between clutch size and egg size in cichlids we focus on how the two traits have evolved in relation to each other in this family. Have the two traits evolved independently of each other, in concert, or in a stepwise or ordered manner where changes in one trait have preceded changes in the other? In particular, we ask how body size may have constrained or facilitated coevolution between clutch size and egg size. This is important since both clutch size and egg size often are strongly related to body size (e.g. reviews by Roff, 1992, 2002; Stearns, 1992). In cichlids, clutch size in particular, but also egg size, is positively related to body size (Kolm *et al.*, 2005). Therefore, we suggest that body size may be involved in the trade-off between clutch and egg size through two possible mechanisms: either as a primary trait driving changes in clutch or egg size, or as a secondary trait that follows changes in the two related traits. Since there is a stronger relationship in cichlids between clutch size and body size than between egg size and body size (Kolm *et al.*, 2005), we predict that body size will be more strongly linked to changes in clutch size than changes in egg size. The maximum likelihood method (Pagel, 1994) thus allows us to test whether changes in body size may facilitate changes in clutch and egg size.

To investigate the evolution of the trade-off between clutch and egg size in cichlids, we used a maximum-likelihood model of correlated evolution (Pagel, 1994, 1997). We compiled a database of quantitative data on clutch size, egg size and body size and used a recently assembled phylogeny (Goodwin *et al.*, unpublished data), based on the latest partial phylogenies among the cichlid fishes. After establishing the most likely ancestral states of these traits, we then tested whether clutch size and egg size have evolved dependent upon each other as well as the most probable evolutionary pathways between ancestral and derived combinations. To detect how changes in body size have affected evolutionary changes in clutch size and egg size, we performed similar pair-wise analyses between clutch size and body size and between egg size and body size.

Methods

Data

Information on clutch size, egg size and body size were collected for as many of the 1400 species within the 222 genera of cichlids as possible. These data originated from Axelrod & Burgess (1988), Brichard (1989), Cichocki (1976), Conkel (1993), Gashagaza (1991), Keenleyside (1991), Kawanabe *et al.* (1997), Konings (1990), Kullander & Nijseen (1989), Linke & Staeck (1994, 1996a, b), Loiselle (1985), Lowe-McConnell (1955, 1959, 1969), Richter (1989), Trewavas *et al.* (1972), Trewavas (1983) and Stawikowski & Werner (1998). Most data on egg sizes originated from Coleman (2002) within Fishbase 2002 (Froese & Pauly, 2002). Since species relationships are currently poorly understood below the genus level in cichlids, we could only resolve phylogenetic relationships down to the genus level. The median of the species values within genera was used to calculate the genus-level values of each variable and all analyses were performed at the genus-level, a procedure which previously has been shown to yield similar results as analyses based on species-data for life histories within this dataset (Kolm *et al.*, 2005).

The comparative method used requires that variables are binary coded (see below). The three continuous variables were therefore divided into two groups. Genera with a value less than or equal to the median value for each variable across all genera were scored as zero and those greater than the median were scored as one (see examples in Cézilly *et al.*, 2000; Krüger & Davies, 2002). Transforming continuous variables into binary variables will inevitably cause a loss of information. However, since the aim of this study is to investigate large-scale patterns of evolution in clutch size, egg size and body size, any loss in information should render our conclusions more conservative. For our variables the medians were as follows; clutch size: 123.5 (range: 12–12000); egg size (diameter): 2.0 mm (range: 1.0–7.0 mm); body size (maximum total length): 16.7 cm (range: 5.25–77 cm).

Phylogenetic relationships

We used the recently constructed composite phylogeny of the cichlid family (Goodwin *et al.*, unpublished data). The phylogeny includes all of the 222 currently known genera. This composite tree over C*ichlidae* could only be constructed very recently due to several new studies that have used extensive molecular and morphological datasets for large sets of taxa (Farias *et al.*, 2001; Klett & Meyer, 2002; Salzburger *et al.*, 2002; Hulsey *et al.*, 2004; Koblmüller *et al.*, 2004). Since there was variation in the number of genera for which data were available, we scaled down the phylogeny to match our database for each specific analysis. For the analyses of ancestral states, the tree for clutch size consisted of 113 genera (i.e. $n = 113$), the tree for egg size had 104 genera, and the tree for body size had 160 genera. For the analyses of dependent correlated evolution of life histories, the tree for the clutch size and egg size analysis consisted of 78 genera, the tree for clutch size and body size had 91 genera and the tree for egg size and body size had 82 genera. Since we used information from a pair of traits on one tree to explain patterns of a pair of traits on another tree, we needed to ensure that the general evolutionary patterns were similar between trees. To do this we performed an additional analysis of correlated evolution for clutch size and body size, using the same tree as used for the analysis of clutch size and egg size (i.e. the largest tree of 91 genera scaled down to the smallest tree of 78 genera). As the general outcome of the analyses did not differ for clutch size and body size between the two trees, we concluded that there were no biases in the results caused by comparing slightly different trees.

Analyses

First, we investigated the current combinations of clutch and egg size among genera. Given the negative correlation between clutch and egg size in cichlids (Kolm *et al.*, 2005), we expected that genera with small clutches and small eggs and large clutches and large eggs would be less common than genera with large clutches and small eggs and *vice versa*.

We then used the programme *Discrete* (Pagel, 1994,1997,1999a,b) to reconstruct the ancestral states of clutch size, egg size and body size, and to investigate whether there was evidence for correlated evolution between life-history traits. *Discrete* is based on a maximum likelihood approach and estimates rates of change between two binary characters without requiring reconstruction of ancestral character states. A likelihood ratio test is used to distinguish between a model that only allows for the independent evolution of two characters (H_0) and a model that enforces correlated evolution (H_1). The likelihood ratio test statistic is $\mathrm{LR} = -2\mathrm{Ln}[H_0/H_1]$, and is asymptotically χ^2 distributed with d.f. = 4 for the comparison for the fit between the independent and the dependent evolutionary models (i.e. test for correlated evolution between each pair of traits). However, since the *Discrete* output provides log-likelihoods, the LR statistic is calculated as twice the difference between the independent and dependent log-likelihoods (i.e. $2 \times [H_0 - H_1]$). We present log-likelihoods throughout the text. Tests of directional character evolution are also possible. We show these potential transitions (q_{ab}) in the form of a flow diagram between trait states (Fig. 1). Each of these transition rates is tested against the null hypothesis that the transition rate is zero. For this test, one performs separate tests for each transition where the transition rate is set to zero, and then compares the resulting model's likelihood to that of the full dependent model. The likelihood ratio test statistic is asymptotically χ^2 distributed with d.f. = 1 for each transition that is estimated. To check that nonsignificant transitions really represented unlikely transitions, we compared the transition rate parameters of such nonsignificant transitions to those that were significant. If a nonsignificant transition had a higher transition rate parameter than that of the significant transition with the lowest transition rate parameter, we considered it to be a likely transition despite its nonsignificance, due to low power. This way of interpreting the statistical output of *Discrete* has been applied by others as a conservative and robust approach (e.g. Rolland *et al.*, 1998). If the transition rate parameter of a nonsignificant transition was lower than that of any significant transition, we interpreted such a transition as highly unlikely. To further estimate the importance of some of the key transitions, we compared transition rate parameters, i.e. the rate of transition from one state to another. Such comparisons follow the same

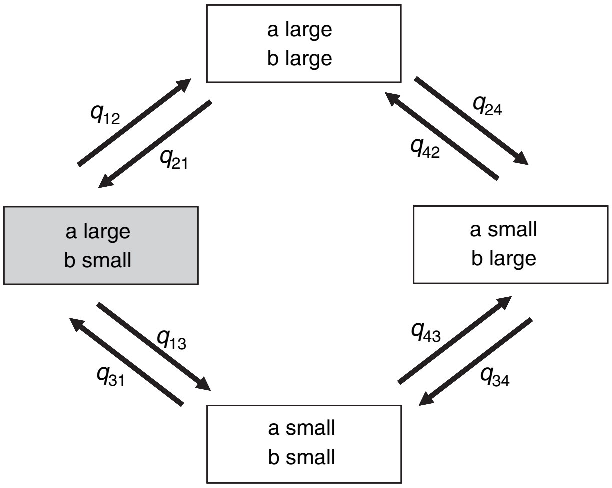

Fig. 1 Flow diagram of the possible transitions of a hypothetical model of dependent correlated evolution of two traits (a and b) that can take two states (large or small). Each possible transition is given by q_{ab} and the hypothetical ancestral combination of traits is shaded in grey.

likelihood ratio test statistics as described above with d.f. = 1.

Analysis of correlated evolution was first performed for the key combination of traits under investigation: clutch size and egg size. Thereafter, we performed similar analyses for the trait combinations clutch size and body size and egg size and body size in order to investigate whether interactions with body size influenced the evolution of the trade-off between clutch size and egg size in cichlids. Although *Discrete* does not allow for direct multivariate comparisons, we can still use this indirect multivariate approach to interpret how body size may have interacted with both clutch size and egg size in shaping the evolution of the two traits in relation to each other.

Since *Discrete* requires a fully branching tree (i.e. nodes with only two descendants), all unknown relationships in the phylogeny were made to be branching but with very short branch lengths (0.0001 units) as recommended by Pagel (1994). All other branch lengths were set to be equal to one, the program option of branch scaling was active and ancestral states were not constrained. None of the results presented were sensitive to differences in the branch length scaling parameter described in Pagel (1994).

Results

As expected, we found that few genera currently have small clutches of small eggs or large clutches of large eggs (Fig. 2). Instead, most genera display the combinations that imply a trade-off between these traits (Fig. 2).

Ancestral states

The ancestral state for clutch size was found to be a large clutch with 99% probability (log-likelihood for ancestral state small clutch = −116.31, log-likelihood for ancestral state large clutch = −111.41, LR = 9.80, d.f. = 1, $P < 0.01$). For egg size, the ancestral state was small eggs with 99% probability (log-likelihood for ancestral state small eggs = −80.74, log-likelihood for ancestral state large eggs = −85.64, LR = 9.79, d.f. = 1, $P < 0.01$). For body size, the ancestral state was a large body with 77% probability (log-likelihood for ancestral state small body = −98.23, log-likelihood for ancestral state large body = −97.02, LR = 2.42, d.f. = 1, $P = 0.12$).

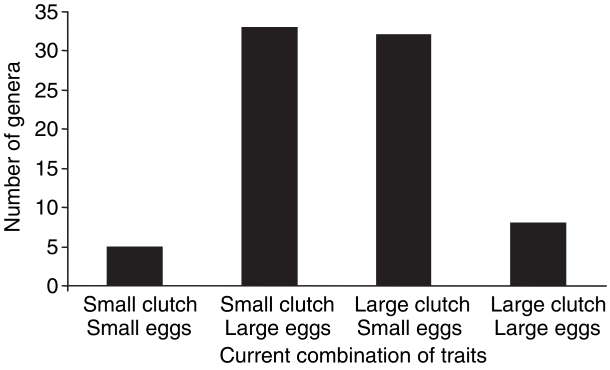

Fig. 2 Overview of the current combinations states of clutch size and egg size among cichlid genera.

Correlated evolution between clutch size and egg size

As predicted, transitions in clutch size and egg size have been strongly correlated. This is shown by the fact that the dependent model provides a better fit than the independent model (the log-likelihood for the independent model was −71.84, compared to −61.66 for the dependent model, LR = 20.4, $P < 0.001$). Therefore, transitions in clutch size and egg size are indeed correlated.

The evolutionary pathways of clutch and egg size in relation to each other are presented in a flow diagram (Fig. 3a). A summary of the test statistics behind all potential transitions can be seen in Table 1a. The most likely route taken from the ancestral state of a large clutch of small eggs to the current, equally common state of a small clutch of large eggs has been via an increase in egg size (q_{12}) followed by a decrease in clutch size (q_{24}). Note that even though transitions q_{24} and q_{21} were not significant in our likelihood model (Table 1a), these transitions are likely since both of their transition rate parameters were much higher ($q_{24} = 0.41$, $q_{21} = 0.49$) than the lowest of the significant transition rate parameters ($q_{24} = 0.07$) (Table 1a). Increases in egg size did not occur in genera with small clutches (q_{34}) (Fig. 3a and Table 1a). After evolving a large clutch of large eggs or a small clutch of small eggs, some genera reverted to the ancestral state of a large clutch of small eggs (q_{21} and q_{31}) (Fig. 3a and Table 1a). Some genera also reverted to a large clutch with large eggs after evolving the common derived state of a small clutch of large eggs (q_{42}) (Fig. 3a and Table 1a).

Transition rate parameters tended to be higher in the direction *from* in comparison *to* the present-day rare combinations of a large clutch with large eggs ($q_{21} > q_{12}$, $q_{24} > q_{42}$, Table 1a and Fig. 3a) and a small clutch with small eggs ($q_{31} > q_{13}$, Table 1a and Fig. 3a), although we only detected a statistically significant difference between transitions from and to a small clutch of small eggs and a large clutch of small eggs ($q_{31} > q_{13}$, LR = 4.5, $P < 0.05$).

Body size in relation to clutch size and egg size

Transitions in both clutch size (Fig. 3b) and egg size (Fig. 3c) were correlated with transitions in body size. Thus, the dependent models provide better fits than the independent models (clutch size and body size: log-likelihood of the independent model was −101.49,

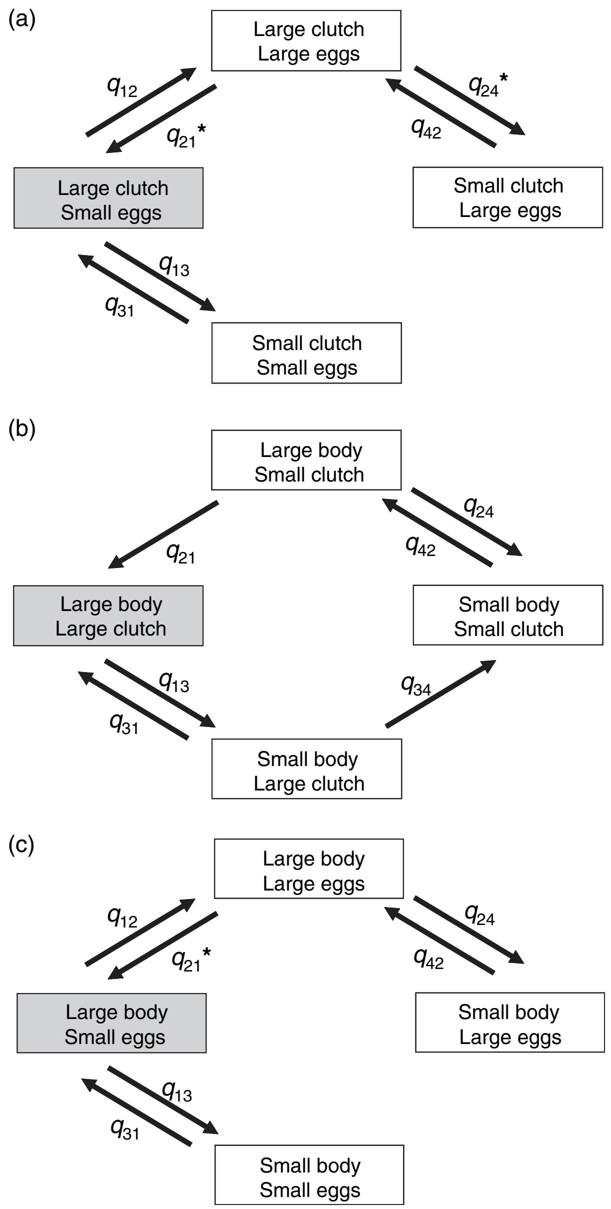

Fig. 3 Flow diagram of the most likely evolutionary transitions between (a) clutch size and egg size (b) clutch size and body size and (c) egg size and body size. The ancestral combination of traits is shaded in grey, and the common current states in cichlids are represented by boldly lined boxes. Solid arrows represents significant evolutionary transitions at the <0.05 significance level and '*' represents those transitions that are likely to have occurred even though they are not significant. See text and Table 1 for details.

Table 1 Comparison of the different potential transitions for the dependent model of correlated evolution between (a) clutch size and egg size, (b) clutch size and body size, and (c) egg size and body size. See Fig. 3 for a visual characterization of the evolutionary transitions.

Transition	Transition rate parameter	*n*	LR	*P*
(a) Clutch size vs. egg size		78		
q_{12}	0.15		14.0	<0.001
q_{13}	0.10		6.6	0.01
q_{21}	0.49*		1.2	0.27
q_{24}	0.41*		0.6	0.42
q_{31}	0.72		7.1	<0.01
q_{34}	<0.001		0.01	0.91
q_{42}	0.07		6.0	0.01
q_{43}	0.03		0.3	0.61
(b) Clutch size vs. body size		91		
q_{12}	0.10		2.6	0.11
q_{13}	0.47		27.0	<0.001
q_{21}	0.27		8.1	<0.01
q_{24}	0.64		15.0	<0.001
q_{31}	0.64		12.4	<0.001
q_{34}	0.46		9.5	<0.01
q_{42}	0.21		21.3	<0.001
q_{43}	<0.0001		0.01	0.94
(c) Egg size vs. body size		82		
q_{12}	0.19		8.0	<0.01
q_{13}	0.69		15.6	<0.001
q_{21}	0.28*		2.2	0.14
q_{24}	0.48		10.7	0.001
q_{31}	1.22		17.8	<0.001
q_{34}	0.03		0.4	0.54
q_{42}	0.18		8.7	<0.01
q_{43}	<0.0001		0.42	0.52

*Represents transitions that are likely to have occurred, despite the fact that they were not statistically significant, due to that their transition rate parameters are higher than those of some significantly significant transition rate parameters within analyses. The transition rate parameters listed are those attained from the full dependent model of evolution.

n represents the number of genera included in each separate combination of traits.

LR, likelihood ratio for each separate transition when comparing the full dependent model to the dependent model when that transition rate parameter was set to 0.

compared to −92.76 for the dependent model, LR = 17.5, $P < 0.01$; egg size and body size: log-likelihood of the independent model was −90.57, compared to −83.82 for the dependent model, LR = 13.1, $P < 0.01$). A summary of the statistics behind all potential transitions for the two combinations of traits can be seen in Table 1b, c.

The most likely route taken from the ancestral state of a large body and clutch size to the current state of a small body and clutch was via a decrease in body size (q_{13}), followed by a reduction in clutch size (q_{34}) (Fig. 3b). Only small-bodied genera reduced clutch size (q_{34}) whereas only large-bodied genera increased clutch size (q_{21}). After evolving a small body with a large clutch some genera reverted to the ancestral states (q_{31}) (Fig. 3b). It was also possible to return to a small body with a small clutch after evolving a large body with a small clutch (q_{24}) (Fig. 3b).

For the evolution of egg size and body size, the derived current pattern is that egg size increases with body size among cichlids, but more weakly so as compared to clutch size (Kolm *et al.*, 2005). Hence, there is a route towards a small body with large eggs from the ancestral state of a large body with small eggs and that route is most likely via an increase in egg size (q_{12}) followed by a decrease in body size (q_{24}) (Fig. 3c). Small-bodied genera did not alter egg size in any direction whereas both increases and decreases in egg size were evident for large-bodied genera (q_{12} and q_{21}) (Fig. 3c). Some genera also reverted to a large body with small eggs after evolving a small body with small eggs (q_{31}) and some also returned to a large body with large eggs after evolving a small body with large eggs (q_{42}).

Discussion

Our results shows that clutch size and egg size have indeed coevolved in cichlids. Furthermore, we have confirmed the prediction that body size may have played a key role in facilitating or constraining transitions in these traits. Specifically, increases in both clutch size and egg size occur predominantly in large-bodied lineages. Furthermore, decreases in clutch size, but not egg size, occur mostly in small-bodied lineages. These are the first findings to indicate the sequence of changes that have produced negative correlations between clutch and egg size among contemporary taxa.

Routes from the past to the present

The most likely route from the ancestral state of a large clutch of small eggs to a small clutch of large eggs was via an initial increase in egg size (Fig. 3a). Then, a decrease in body size occurred prior to the decrease in clutch size (Fig. 3b). This evolutionary scenario is illustrated in Fig. 4a. This body size interaction may have led to the positive relationship between clutch size and body size in cichlids (Kolm *et al.*, 2005) as well as many other taxa (e.g. Roff, 1992; Stearns, 1992).

While the data show that cichlids have been following the route indicated in Fig. 4a, it is worth considering why they did not take the alternative route in Fig. 4b, namely towards small clutches of large eggs via a decrease in clutch followed by an increase in egg size. We believe that interactions with body size are again likely to be responsible. The data show that the first step to a small clutch of small eggs is most likely following a decrease in body size (Fig. 3b). But this transition closes the pathway to large eggs (transition 2 in Fig. 4b) since only large genera have increased egg size (Fig. 3c). We are therefore left with the situation whereby those small-bodied fish that have large eggs have arrived at this condition through reduction in body size rather than increases in egg size. Perhaps it has been 'easier' for such species to accommodate large eggs if they have retained adaptations from their larger ancestors, rather than evolving them as novel traits. For example, in cichlids, mouthbrooding species tend to have larger eggs and mouths than egg guarders (Goodwin *et al.*, unpublished data). If decreases in body size were not associated with comparable decreases in mouth size in mouthbrooders, this could explain how small genera with large eggs have evolved from large genera.

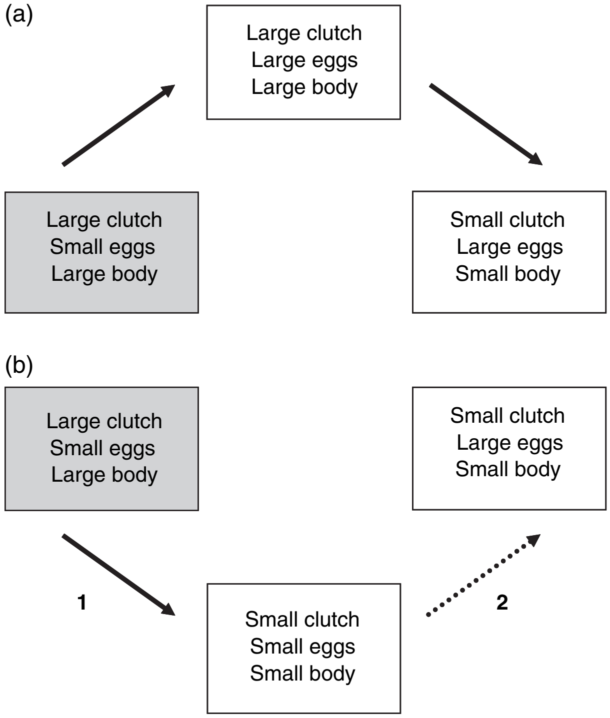

Fig. 4 Flow diagram of (a) the most likely pathway from the ancestral state of a large bodied cichlid with a large clutch of small eggs to the common current state of a small bodied cichlid with a small clutch of large eggs and (b) the other potential but unlikely pathway between the ancestral state of a large bodied cichlid with a large clutch of small eggs to the common current state of a small bodied cichlid with a small clutch of large eggs. In (b) the two necessary transitions (1 and 2) are highlighted: 1 is possible but 2 (dotted arrow) is unlikely since only large genera have made the transition from small to large eggs. See text for details.

Towards the negative relationship between clutch size and egg size

The rarity of cichlid genera that produce large clutches of large eggs or small clutches of small eggs is generally attributed to trade-offs in investments between size and number of eggs by females (Roff, 1992, 2002; Stearns, 1992). Our empirical results also show that cichlids through evolution have moved away from these states since transitions *from* the rare states have been more common than transitions *to* these rare states.

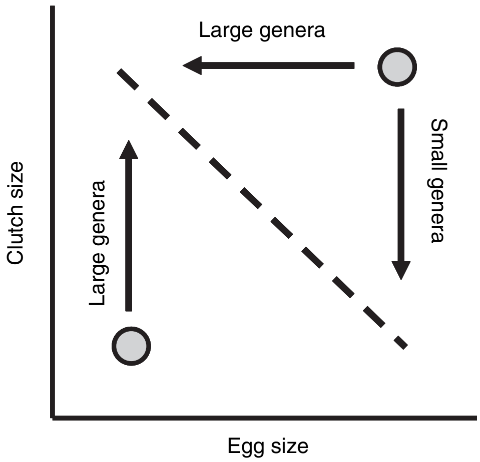

Fig. 5 Size-dependent pathways from unstable states of clutch size and egg size to the negative relationship between the two traits in cichlids. For genera with large clutches of large eggs (top right corner), large-bodied genera have decreased egg size whereas small-bodied genera have decreased clutch size. For genera with small clutches of small eggs (low left corner), large-bodied genera have increased clutch size. See text for details.

Interestingly, interactions with body size have been important in moving away from these rare combinations of traits and towards the classical negative correlation between clutch and egg size (Fig. 5). First, consider species that produce both large eggs and clutches (top right-hand corner Fig. 5). Large-bodied genera have reduced their egg sizes but not clutch sizes (Fig. 3), thereby reaching the top left corner of the clutch/egg space in Fig. 5. If large enough, genera may thus cross a threshold where the relationship between clutch size and body size thus render the 'clutch size' strategy more beneficial (e.g. Bagenal, 1966; Wootton, 1979; Roff, 2002). Conversely, small-bodied taxa have reduced clutch size but not egg size, reaching the bottom right corner of Fig. 5. Many such species may be mouthbrooders, as these tend to be smaller than egg guarders (Goodwin *et al.*, unpublished data), and produce smaller clutches of larger eggs. Hence, the evolution of mouthbrooding, matched with a small clutch of large eggs, may have been a way for smaller sized cichlids to increase their reproductive output under such a size constraint. Now consider species that produce small clutches and eggs. Again, size appears to predict the change as large genera with this combination of traits have not changed egg size, but have increased clutch size (Fig. 3), thereby reaching the top left corner of the clutch/egg space in Fig. 5. Together, these observations suggest that the fitness optimum of large genera is more dependent on number of offspring as compared to small genera where the fitness optimum is more dependent on the quality of the offspring. This suggest that cichlids have evolved in the opposite way of the fast-slow continuum common in mammals, where large species generally have small litters of large offspring and small species have large litters of small offspring (e.g. Harvey & Purvis, 1999). This is likely an effect of the indeterminate growth of fishes, along with the stronger relationship between clutch size and body size as compared to egg size and body size (Kolm *et al.*, 2005). Note that the scenarios depicted in Fig. 5 show the main biases, reflecting the dichotomous nature of the analyses, and are not meant to imply that there have never been joint transitions in both clutch and egg size.

The interaction between egg traits and body size may also have been important in other systems in shaping the trade-off between clutch size and egg size. This is likely because body size is positively related to clutch size in many taxa (see review by Roff, 2002), although, as mentioned above, this relationship is less clear in mammals (Roff, 2002) and across major lineages of birds (Bennett & Owens, 2002). For egg size, the evidence is more unanimous since most taxa show a positive relationship between egg size and body size, although the strength of this relationship may vary (Roff, 1992; Bennett & Owens, 2002). It would be interesting to perform studies similar to the present one on these other taxa, especially for those groups of mammals and birds that lack a positive relationship between clutch size and body size, to investigate whether the trade-off between clutch size and egg size has produced different routes of evolution of these traits in relation to body size in these taxa.

To conclude, we have used a novel approach to investigate how the widespread negative correlation between clutch size and egg size has been produced via correlated evolution between these traits. Moreover, we show that body size and its transitions have determined the directions of change in these traits. We suggest using these methods for other taxa in order to fully understand the evolution of this trade-off.

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