

Evolution of egg dummies in Tanganyikan cichlid fishes: the roles of parental care and sexual selection

M. AMCOFF*, A. GONZALEZ-VOYER† & N. KOLM*

*Animal Ecology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

†Conservation and Evolutionary Genetics Group, Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas (EBD-CSIC), Sevilla, Spain

Keywords:

BayesTraits;
egg dummies;
phenotypic evolution;
sexual selection;
signal evolution;
sperm competition.

Abstract

Sexual selection has been suggested to be an important driver of speciation in cichlid fishes of the Great Lakes of Africa, and the presence of male egg dummies is proposed to have played a key role. Here, we investigate how mouthbrooding and egg dummies have evolved in Tanganyikan cichlids, the lineage which seeded the other African radiations, with a special emphasis on the egg dummies. Using modern phylogenetic comparative analyses and a phylogeny including 86% of the 200 described species, we provide formal evidence demonstrating correlated evolution between mouthbrooding and egg dummies in Tanganyikan cichlids. These results concur with existing evidence, suggesting that egg dummies have evolved through sensory exploitation. We also demonstrate that there is a strong evolutionary correlation between the presence of egg dummies and both pre- and post-copulatory sexual selection. Moreover, egg dummy evolution was contingent on the intensity of pre- and post-copulatory sexual selection in Tanganyikan cichlids. In sum, our results provide evidence supporting the hypothesis of egg dummies evolving through sensory exploitation and highlight the role of sexual selection in favouring the evolution and maintenance of this trait.

Introduction

The cichlid fishes of the Great Lakes of East Africa (Lakes Victoria, Malawi and Tanganyika) are one of the most famous examples of rapid speciation and adaptive radiation (Kocher, 2004; Seehausen, 2006). Within a very recent evolutionary past (5–6 million years in Lake Tanganyika [Koblmüller *et al.*, 2008], 1–2 million and 200 000 years in Lake Malawi and Victoria, respectively [Verheyen *et al.*, 2003; Salzburger & Meyer, 2004] but see Genner *et al.*, 2007), almost 2000 cichlid species evolved in these lakes (Snoeks, 2000; Kocher, 2004). Of these, at least 200 cichlids (almost all of them endemics) reside in Lake Tanganyika, the oldest of the East African rift lakes, which seeded the radiations of the two other lakes (Fryer & Iles, 1972; Nishida, 1991; Salzburger *et al.*, 2002, 2005). The Tanganyikan cichlids have been divided into 16 different tribes (Koblmüller

et al., 2008 but see Poll, 1986; Salzburger *et al.*, 2002; Takahashi, 2003), and they display a remarkable array of brood care patterns, ecological niche specializations and phenotypes (Fryer & Iles, 1972; Chakrabarty, 2005; Young *et al.*, 2009). In contrast, almost all the cichlids in Lakes Malawi and Victoria belong to the most species-rich tribe Haplochromini (Turner *et al.*, 2001; Salzburger *et al.*, 2005). The exceptional species richness in the haplochromines may be explained by two particular attributes that almost all species in this tribe possess: parental care in the form of maternal mouthbrooding and the presence of egg dummies (Oppenheimer, 1970; Goldschmidt & de Visser, 1990; Salzburger *et al.*, 2005; but see Wagner *et al.*, 2012).

Females of mouthbrooding species pick up the eggs into their mouths and do not release them until the fry are fully developed several weeks later (Oppenheimer, 1970; Fryer & Iles, 1972; Kuwamura, 1997; Sefc, 2011). In some species, males also contribute to parental care, for example, by mouthbrooding the fry or by offering protection after the fry have been released, but in the majority of the species, parental care is exclusively provided by the female (Kuwamura, 1986; Sefc, 2011).

Correspondence: Mirjam Amcoff, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden.
Tel.: +46 18 471 6495; fax: +46 18 471 6484;
e-mail: mirjam.amcoff@ebc.uu.se

This strong skew in reproductive investment may set the stage for intense sexual selection in mouthbrooding species (Oppenheimer, 1970). To exert mate choice, females can use indicators of mate quality such as morphology, behaviour or territory size prior to mating (Andersson, 1994) or post-mating through cryptic female choice (Eberhard, 1996).

During spawning, before the female has picked up the eggs in her mouth, the eggs are vulnerable to egg predation by con- and heterospecifics (Mrowka, 1987b; Nshombo, 1991). This led Wickler (1962) to suggest that females were selected to pick up the eggs so fast that males would sometimes not have time to fertilize them. Males of many mouthbrooding cichlids bear egg-like markings known as 'eggspots' or 'egg dummies' on the anal fin (Wickler, 1962; Salzburger *et al.*, 2005). During courtship, females of some species nibble the males' genital area where the egg dummies are located seemingly trying to pick them up into their mouth (Wickler, 1962; Salzburger *et al.*, 2005). This behaviour led researchers to suggest that egg dummies are mistaken for real eggs by the females and that if the males release sperm when the females are nibbling at the egg dummies, it would be inhaled by the female and the eggs held in her mouth would be fertilized (Wickler, 1962; Coeckelberghs, 1976; Salzburger *et al.*, 2005). Indeed, previous work has shown that fertilization happens inside the mouth of some species (Mrowka, 1987a).

Other, not mutually exclusive, theories have been offered to explain the evolution of egg dummies, but one having received recent support is that of evolution through sensory exploitation, which forms an extension of Wickler's egg-mimicry theory (Tobler, 2006). The sensory exploitation hypothesis states that a sensory bias under natural selection may, as a side effect, generate a preference in a sexual selection context (West-Eberhard, 1984; Ryan & Rand, 1990; Proctor, 1991). The main prediction of the sensory exploitation hypothesis is that the preference (picking up eggs into the mouth for brooding) preceded the trait (egg dummies) in evolutionary time (Basolo, 1990). Egger *et al.* (2011) provided evidence in favour of the sensory exploitation hypothesis by showing that female *Pseudocrenilabrus multicolour*, a mouthbrooding East African riverine species (Mrowka, 1987c), prefer males with an artificial egg dummy over natural males without egg dummies, thus showing that there is a pre-existing preference for egg dummies in this species. Furthermore, the authors showed, in a colour-dot preference test, that an additional 11 species (substrate guarding and mouthbrooding Tanganyikan cichlids with and without egg dummies) showed preferences for egg dummy-related colours (yellow, orange and red; Egger *et al.*, 2011). Within the haplochromine group of cichlids, mouthbrooding has been shown to precede the presence of egg dummies in evolutionary time (Salzburger *et al.*, 2005,

2007). However, as yet, no study has demonstrated whether the two traits evolved in a correlated fashion.

We used the behavioural diversity offered by the Lake Tanganyika cichlid assemblage to further investigate egg dummy evolution and maintenance. There are both mouthbrooding and substrate guarding cichlids within Lake Tanganyika, and parental care type ranges from strictly maternal care with little paternal investment to biparental care and cooperative breeding (Kuwamura, 1986; Salzburger *et al.*, 2002). Unlike the other East African Lakes where only one type of egg dummy is found, there are many different varieties of egg dummies within the Tanganyikan cichlids (Tobler, 2006). These different types of egg dummies include egg-like markings of varying elaboration and number on the anal fin in the Haplochromini and Tropheini as well as in some species of the *Bathybates* and *Callochromis* genera (Salzburger *et al.*, 2007). In addition, the pelvic fins in species of the *Cyprichromis*, *Cyathopharynx* and *Ophthalmotilapia* genera sometimes have pigmented tips (Salzburger *et al.*, 2005; Tobler, 2006). Although the function of the egg dummies has only been investigated in a handful of species, they appear to be used in the same manner among species: the males vibrate the pigmented tips of the pelvic fins or the egg dummies on their anal fins in front of the females and females of many species perform the 'nibbling' behaviour towards the egg dummies during courtship (Tropheini: Coeckelberghs, 1976; Kuwamura, 1987; Haplochromini: Fernald & Hirata, 1977; Ectodini: Immler & Taborsky, 2009; Cyprichromini: Takahashi & Hori, 2006). This suggests that the egg dummies potentially play a role in mate choice at least during close-range courtship but potentially also from a distance. The apparent similar function of different types of egg dummies has also been proposed to form a case of parallel evolution in the cichlids of East Africa (Sturmbauer *et al.*, 2003; Salzburger *et al.*, 2007; Salzburger, 2009).

The evolutionary origin of the egg dummies may be different from their current function. Although it is possible that sensory exploitation is still at work, other selective forces may be acting concurrently or may even have replaced the original evolutionary driver entirely (Garcia & Ramirez, 2005). Precopulatory sexual selection in the form of, for example, mate choice has been suggested to be a strong evolutionary force behind the evolution and maintenance of egg dummies (e.g. Goldschmidt, 1991; Tobler, 2006). There is some evidence that egg dummies may function as a quality indicator because egg dummy number and onset of ontogeny correlate positively with age, condition and dominance status in cichlid fishes (e.g. Balon, 1977; Heule & Salzburger, 2011; Lehtonen & Meyer, 2011; Henning & Meyer, 2012; Theis *et al.*, 2012). However, specific tests of female preference for the egg dummies have produced ambiguous results with females of some species preferring males with egg dummies, whereas

others prefer males without egg dummies (Hert, 1989, 1991; Couldridge & Alexander, 2001; Couldridge, 2002; Henning & Meyer, 2012; Theis *et al.*, 2012). Post-copulatory sexual selection in the form of sperm competition has been proposed to play a major role in sexual selection (Birkhead & Møller, 1998; Møller & Ninni, 1998), but is a previously unexplored avenue in the context of egg dummy evolution. Here, we set out to investigate whether precopulatory sexual selection (quantified using information about mating system and sexual dimorphism) and post-copulatory sexual selection (quantified as risk of sperm competition) may be involved in the current maintenance of egg dummies.

First, we used phylogenetic comparative analyses of evolutionary contingency (Pagel & Meade, 2006) to determine whether mouthbrooding and egg dummies evolved in a correlated and dependent fashion, in accordance with the sensory exploitation hypothesis. Next, we investigated whether an association exists between presence/absence of egg dummies and intensity of pre- and post-copulatory sexual selection. If egg dummies function as a precopulatory sexually selected signal or to reduce the risk of sperm competition, we predicted that they would occur mainly in species with high intensity of sexual selection. Finally, we investigated whether an increase in sexual selection was contingent on the presence of egg dummies and whether egg dummy evolution was dependent on the intensity of sexual selection. We predicted that, if the evolution of egg dummies was influenced by sexual selection, the occurrence of egg dummies would be correlated with an increase in the intensity of precopulatory sexual selection and/or risk of sperm competition. Under the same scenario, we also predicted that the evolution of egg dummies would be contingent on an increase in either form of sexual selection.

Our results show that the egg dummies have evolved in a correlated fashion with mouthbrooding, which supports previous studies suggesting evolution through sensory exploitation. Furthermore, we demonstrate that the evolution of egg dummies is contingent on the intensity of both pre- and post-copulatory sexual selection.

Materials and methods

In this study, we focus on the group of species residing in Lake Tanganyika. The Malawi and Victoria radiations were seeded by haplochromine Tanganyikan cichlids (Salzburger *et al.*, 2002, 2007), and except for a small number of species, all haplochromines exhibit egg dummies. The exceptions are derived species that lost them and a few basal species that presumably never had them (Salzburger *et al.*, 2002, 2005; Egger *et al.*, 2011). Thus, it is highly likely that the egg dummies emerged in the Tanganyikan lineage, and not including Malawi or Victoria cichlids is unlikely to influence the results

due to their limited variation in the traits of interest. Furthermore, we chose to consider only lacustrine species, that is, species present in the actual lake, because the selective pressures acting on riverine species may differ from those experienced by lacustrine species, and several cichlid species currently found in the surrounding rivers trace their ancestry back to Lake Tanganyika (Salzburger *et al.*, 2002). Phylogenetic reconstructions suggest that the lacustrine radiation of the Tanganyikan lineage was seeded by six or eight lineages, which originated from cladogenesis events prior to lake formation (Salzburger *et al.*, 2002). Hence, our comparative analyses are likely to involve an evolutionary time frame spanning early proto-lake conditions to the present. Such circumstances limit our ability to determine whether traits evolved within or prior to lake formation, but do not influence the results of analyses of correlated evolution or contingency.

Data on egg dummies

Because our interest was to gain better understanding of the large-scale evolution of egg dummies across all Tanganyikan cichlids, we included species with all types of egg dummies in our analyses (regardless of their location and colour variation). We defined egg dummies following Tobler's (2006) broad sense description and included species with more or less well-defined orange, yellow or red pigmented spots or areas located on the anal fin or pelvic fin tips. For the analysis of correlated evolution between form of parental care (mouthbrooding or substrate guarding) and egg dummy presence/absence, we collected data from reference books, primary publications and original descriptions (see Table S1 in the Supporting Information). To ensure accuracy, an independent cichlid expert (Erwin Schraml), who was unaware of the hypothesis we wished to test, confirmed our egg dummy classifications. Both form of parental care and egg dummy presence/absence were coded as dichotomous variables, as required by the analysis (Pagel & Meade, 2006).

Data on precopulatory sexual selection

Having found a strong significant association between egg dummies and mouthbrooding (see Results) and because, as far as we know, no substrate guarding species with egg dummies has ever been described, we restricted our analyses of the relationship between egg dummy presence/absence and intensity of precopulatory sexual selection to mouthbrooding species. To construct a robust proxy for precopulatory sexual selection, we combined data on mating system and sexual dichromatism for 62 mouthbrooding species (Gonzalez-Voyer *et al.*, 2008). The social mating system within the mouthbrooding cichlids of Lake Tanganyika can largely be divided into lekking species, where the males keep

mating territories that the females visit for spawning, harem-forming and pair-bonding species (Sefc, 2011). Information about mating system was collected from primary publications (see Table S2), and the collected information was verified by five cichlid experts (Masanori Kohda, Tetsumi Takahashi, Satoshi Awata, Tetsuo Kuwamura and Tomoki Sunobe). The species were given a two-level rank (present/absent) of sexual dichromatism following the definition used by Gonzalez-Voyer & Kolm (2011). This was done by a cichlid expert (Heinz Büscher) who was unaware of the hypothesis being tested. These data were then combined into a rank dividing the species into low, moderate and high intensity of precopulatory sexual selection.

There are inherent difficulties associated with assigning species to different sexual selection categories when little is known about the mating behaviour of many species. To deal with this difficulty and to minimize the amount of error associated with assigning species to different ranks, we only used three broad categories of precopulatory sexual selection in our correlation analysis and two categories in our contingency analysis.

We ranked all species ($n = 20$) with a monogamous mating system as having a low intensity of sexual selection (Emlen & Oring, 1977). To divide the species with different polygynous mating systems into moderate and high intensity of sexual selection, we used the information about sexual dichromatism. Species displaying a lekking or harem-forming mating system were ranked to have moderate intensity of sexual selection if no sexual dichromatism was present ($n = 9$) and high intensity of sexual selection if sexual dichromatism was present ($n = 33$; Table S2).

Data on post-copulatory sexual selection using sperm competition as a proxy

As with precopulatory sexual selection, we tested the relationship between egg dummy presence/absence and risk of sperm competition using only mouthbrooding species. Because the genetic mating system has not been investigated in more than a handful of the Lake Tanganyika mouthbrooding cichlids (Taylor *et al.*, 2003; Egger *et al.*, 2006; Sefc *et al.*, 2009; Haesler *et al.*, 2011; Takahashi *et al.*, 2011), we followed Balshine *et al.* (2001) and Fitzpatrick *et al.* (2009) and used social mating system in combination with detailed descriptions of mating behaviour as a proxy for sperm competition. Based on detailed descriptions of the spawning behaviour in primary publications, we noted down whether females of lekking species had been observed to visit several males and whether sneaking behaviour was present.

Five cichlid experts (Masanori Kohda, Tetsumi Takahashi, Satoshi Awata, Tetsuo Kuwamura and Tomoki Sunobe) verified the accuracy of our data on

mating behaviour. These data were then combined into a sperm competition rank dividing the species into low, moderate and high risk of sperm competition. The species were ranked independently by two of the authors (M. A. and A. G.-V.), and the two ranks were identical. Finally, two independent scientists familiar with the cichlid system, but who were unaware of the hypothesis being tested (Masahito Tsuboi and Alexander Kotrschal), confirmed the sperm competition rank. Worth noting here is that, with the exception of *Tropheus moorii*, our ranks of sperm competition overlapped completely with the ranks included in previous analyses of sperm competition evolution in the Tanganyikan cichlids (Balshine *et al.*, 2001; Fitzpatrick *et al.*, 2009).

We ranked species as presenting low risk of sperm competition when females mate with one or two males at most, and thus, the sperm of one male risks coming into contact with that of no more than one more male. We placed the 20 species that have been described as monogamous (see Table S2) in this category, two of which have been shown to be genetically monogamous (*Eretmodus cyanostictus* [Taylor *et al.*, 2003] and *Xenotilapia rotundiventralis* [Takahashi *et al.*, 2011]). *T. moorii* was also included in this category, because results from previous work have shown that it is genetically monogamous even though it is a lekking species (Egger *et al.*, 2006). In this group, we also placed five harem-forming species with extensive mate guarding because this male behaviour reduces the risk of multiple matings (Table S2).

In species where females sequentially visit several males, sperm competition has been shown to occur and eggs can be fertilized by a male, even if no eggs were spawned in that particular males' territory (Haesler *et al.*, 2011). Therefore, species were ranked as presenting moderate risk of sperm competition when females visit and may mate with more than two males and there is a time lag separating the matings. Thus, in this category, the sperm of one male risks coming into contact with sperm from more than two males in a sequential fashion. The genetic mating system has been shown to correlate with social mating system in a range of taxa, and multiple paternity is common in lekking cichlids (Höglund & Alatalo, 1995; Parker & Kornfield, 1996; Kellogg *et al.*, 1995, 1998 but see Egger *et al.*, 2006). We therefore placed all lekking species (27 species; see below and Table S2) in the moderate risk of sperm competition category except those in which sneaking behaviour has been observed. In eight of these species, females have been observed to subsequently visit several males for spawning.

Sneaking behaviour has been observed within the Lake Tanganyika cichlids (Sefc, 2011). The male that is actively courting the female may have a reproductive advantage over late-arriving sneakers due to the latency between sperm transfers (Taborsky, 1998). There is some variation in latency between sperm

transfers within sneaking species as well. For instance, sneakers of some species rush in to release sperm while the resident male is chasing off other intruders (e.g. *Ophthalmotilapia ventralis* [Haesler *et al.*, 2009]), whereas others spawn simultaneously with the resident male (e.g. *Pseudosimochromis curvifrons* [Kuwamura, 1987]). Nonetheless, sneakers should have an advantage over any male the female subsequently mates with, as the latency to sperm deposition in the latter case is even greater. Hence, sperm competition is expected to be more intense in species where sneakers are present than in those where the females only visit males in a sequential fashion. We therefore categorized species as presenting high risk of sperm competition when the sperm of one male risks coming into contact with that of another male almost simultaneously. This occurs in nine species where sneakers are present including one harem-forming species (*Ctenochromis horei*; Ochi, 1993; Sefc *et al.*, 2009).

Because the pre- and post-copulatory scores were both based partially on social mating system, we expected these scores to be correlated. Nonetheless, the two scores provide slightly different information. For instance, in the absence of sneakers, the harem-forming species are expected to have a low risk of sperm competition due to the mate guarding strategy of the males (Fig. 1). These same species are expected to have a moderate to high level of precopulatory sexual selection due to the increased variance in male mating success when a few males monopolize several females. The additional information (sexual dichromatism and mating behaviour, respectively) further distinguishes the two scores, which thus offer slightly different pieces of information about sexual selection.

Phylogeny

We reconstructed a molecular phylogeny of the Lake Tanganyika cichlids to date representing 86% (171 species) of the 200 described species (or 68% of the estimated total of 250 species; Koblmüller *et al.*, 2008; Takahashi & Koblmüller, 2011) including representative species from all 16 cichlid tribes (Koblmüller *et al.*, 2008). We used three mitochondrial genes, a noncoding control region (d-loop) and two coding genes: NADH2 and the more conserved cytochrome b (Koblmüller *et al.*, 2005). The variation in evolutionary constraints acting on these three genes allowed us to address splits among more closely related taxa as well as in the deeper nodes of the tree (Kocher *et al.*, 1995; Duftner *et al.*, 2005; Koblmüller *et al.*, 2005). These genes have previously proven useful for shedding light on species-level relationships in Lake Tanganyika cichlids (e.g. Kocher *et al.*, 1995; Salzburger *et al.*, 2002; Koblmüller *et al.*, 2005; Day *et al.*, 2007). Sequences were downloaded from GenBank for the Tanganyikan species (see Table S3 for accession numbers) and for the West African cichlid

Heterochromis multidens, which was used as the outgroup (Farias *et al.*, 2000). Sequences were aligned using MAFFT (Katoh *et al.*, 2002), and minor adjustments were done manually in BioEdit (Hall, 1999). We constructed one genealogy for each gene in RAxML (Stamatakis, 2006) under a general time reversible (GTR) model of substitution with a Gamma model of rate of heterogeneity (Stamatakis *et al.*, 2008). For many species, several sequences were available for each gene. In such cases, to minimize biases in the phylogenetic reconstruction due to misidentification or highly divergent sequences, one sequence per species was selected from those clustering together with sequences from the same species, avoiding any sequence that differed from the others in its position in the genealogy. The matrices were concatenated creating a matrix of 3080 bp (NADH2: 1047 bp; cytochrome b: 1146 bp; control region: 886 bp). A GTR model of substitution with a proportion of invariant sites and gamma distribution of rate heterogeneity was found to present the best fit for the three genes using jModelTest (Guindon & Gascuel, 2003; Posada, 2008). We tested for saturation in the third codon position of the two coding genes using DAMBE (Xia, 2001) and found both genes presented evidence of saturation (see Figs S1 and S2). The phylogenetic tree was reconstructed under Bayesian inference (BI) in MrBayes, version 3.1.2 (Ronquist & Huelsenbeck, 2003). To account for saturation in the third codon, we ran an analysis with a partition by codon for the two coding genes. We ran two separate runs with 6 chains in each run. The analysis was implemented in the CIPRES Portal (Miller *et al.*, 2009). The chains were run for 22 million iterations with a relative burnin of 25% (5.5 million iterations) and a sampling period of 1000 leaving 16 500 sampled trees post-burnin. Model conversion was investigated by visual inspection of diagnostics plots in AWTY and by ensuring that the average standard deviation of split frequencies was < 0.01 (Wilgenbusch *et al.*, 2004).

Phylogenetic comparative analyses

We used the BayesDiscrete method in the program BayesTraits to test for correlated evolution and contingency between form of care and presence/absence of egg dummies (Pagel & Meade, 2006). The BayesDiscrete model describes changes in one or two dichotomous traits over branches of a phylogenetic tree via a continuous-time Markov process (Pagel *et al.*, 2004; Pagel & Meade, 2006). BayesTraits implements a Bayesian Markov chain Monte Carlo method that allows controlling simultaneously for uncertainty in the model of evolution as well as phylogenetic uncertainty, by including a random subset of phylogenies rather than only the consensus tree (Pagel & Meade, 2006). Here, we included a subset of 500 trees drawn from the converged Markov tree inference chain from the MrBayes analyses. All species for which we lacked information about parental

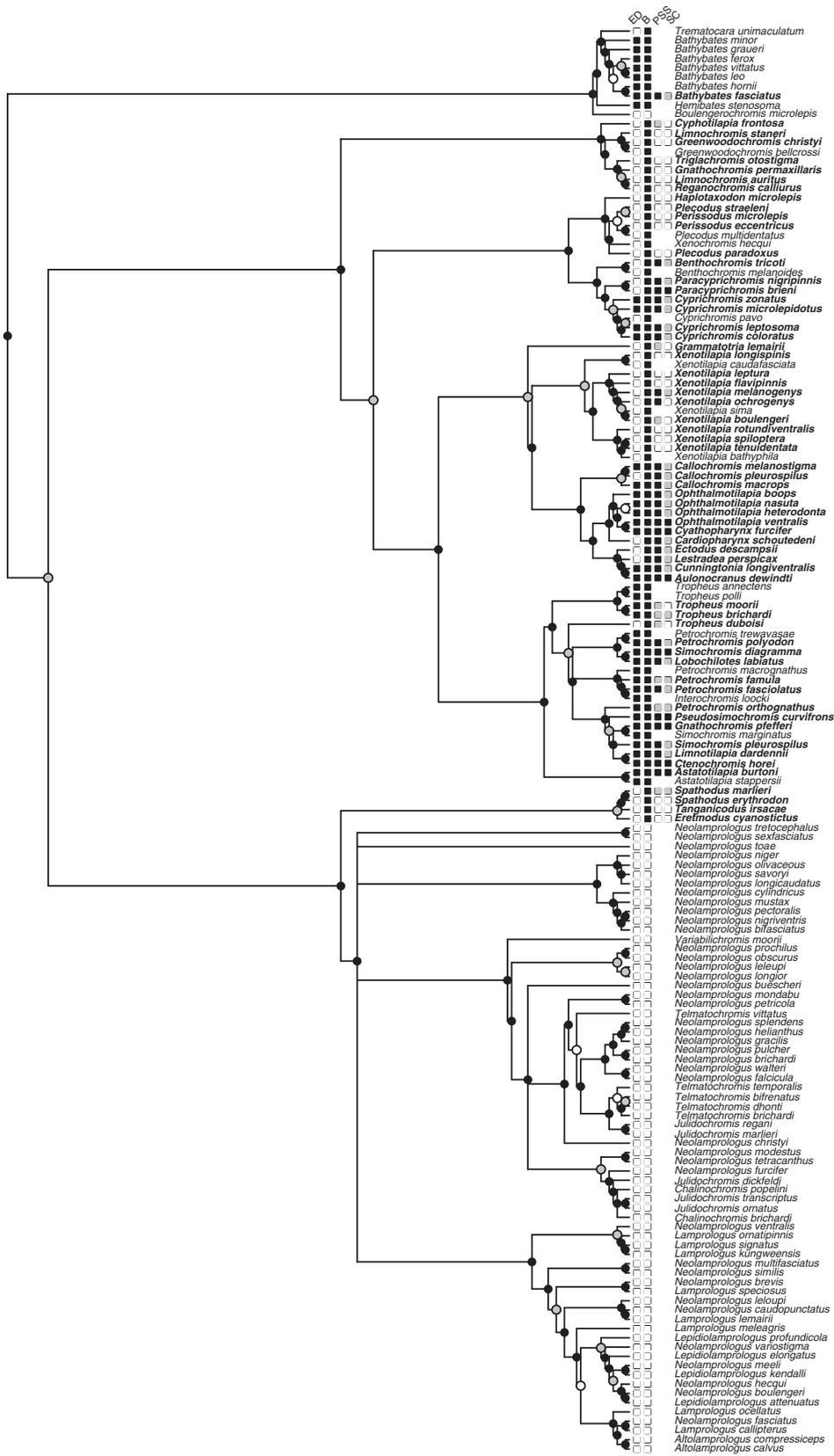


Fig. 1 Consensus phylogenetic tree of the 154 species included in the analysis of correlated evolution between form of parental care (B) and egg dummy presence/absence (ED). Highlighted in bold are the 62 species included in the analysis of correlated evolution between egg dummy presence/absence, intensity of precopulatory sexual selection (PSS) and risk of sperm competition (SC). Node labels indicate posterior probabilities; white circles < 0.60, grey circles, 0.61–0.90 and black circles > 0.90. Shown in the figure are species-specific trait states for egg dummies (presence: black squares; absence: white squares), form of parental care (mouthbrooding: black squares; substrate guarding: white squares), intensity of precopulatory sexual selection (low: white squares; moderate: grey squares; high: black squares) and risk of sperm competition (low risk: white squares; moderate risk: grey squares; high risk: black squares). Branch lengths are in number of expected substitutions.

care and/or egg dummy presence/absence were removed leaving trees with 154 tips (Fig. 1). We were interested in testing whether mouthbrooding and egg dummies have evolved in a correlated fashion and whether egg dummy evolution is contingent on mouthbrooding, that is, whether the evolution from absence to presence of egg dummies depended on the form of brooding behaviour (substrate guarding/mouthbrooding). The dependence of two traits can be investigated by comparing one model, where the traits evolve independently of one another, with another where they are allowed to evolve in a correlated fashion (Pagel & Meade, 2006; Gonzalez-Voyer *et al.*, 2008). These two models can then be compared using the log-BayesFactor test ($2(\log[\text{harmonic mean}(\text{dependent model})] - \log[\text{harmonic mean}(\text{independent model})])$; Pagel & Meade, 2006); any positive value is evidence in favour of the dependent model. We ran independent and dependent models with gamma or exponentially distributed priors. The prior means and variance were seeded from a hyper prior with a uniform distribution (range, 0–10; see Supporting Information, Materials and methods for more details), and the results were insensitive to the choice of prior. We ran the chains for 5 050 000 iterations with a burnin of 50 000 and a sample period of 500 leaving a sample of 10 000 iterations. To estimate the likelihood of evolutionary transitions between trait state combinations (Z-score), for each transition, we calculated the proportion of all samples from the posterior distribution in which the transition value was set to 0, that is, it does not occur, following Pagel & Meade (2006); (see also Gonzalez-Voyer *et al.*, 2008).

We tested whether the relative number of species presenting egg dummies (response variable) increased with increasing intensity of precopulatory sexual selection (explanatory variable) using a phylogenetically controlled multivariate generalized linear mixed model with Markov chain Monte Carlo estimation using the MCMCglmm package (v. 2.06; Hadfield, 2010) in R (v. 2.10.1; R Development Core Team, 2005). In these analyses, presence/absence of egg dummies was set as a binomial response with two levels (present/absent), and intensity of sexual selection was set as a categorical predictor with three levels (low, moderate and high) in a model with a binomial distribution and a logit link function (see Hadfield, 2010; Hadfield & Nakagawa,

2010 and Supporting Information, Materials and methods, for details). Again the tree was pruned removing all substrate guarders and those mouthbrooders for which we lacked information about either egg dummy presence/absence or intensity of sexual selection, leaving a tree with 62 tips. Parameter estimates are the modes from the posterior distributions (β), the 95% lower and upper credible intervals (CI) and *P*-values. As expected, the pre- and post-copulatory sexual selection ranks were strongly correlated ($r = 0.81$, d.f. = 60, $P < 0.001$). Therefore, the correlation between relative number of species presenting egg dummies (response variable) and increasing level of pre- and post-copulatory sexual selection (explanatory variables with three levels: low, moderate and high) were investigated in separate, but identical analyses.

We used the BayesDiscrete method to test for contingency in the evolution of intensity of precopulatory sexual selection and presence/absence of egg dummies (Pagel & Meade, 2006), that is, whether the evolution from absence to presence of egg dummies depended on the intensity of sexual selection (low/high). To do this, we dichotomized the three-level sexual selection rank, as required by the analyses, by combining the moderate and high intensity of sexual selection categories into one single category representing high intensity of sexual selection. The dependent and independent models were run with gamma or exponentially distributed priors. The prior means and variance were seeded from a hyper prior with a uniform distribution (range, 0–10; see Supporting Information, Materials and methods for more details), and the results were insensitive to the choice of prior. We ran the chains for 5 050 000 iterations with a burnin of 50 000 and a sample period of 500 leaving a sample of 10 000 iterations. In a similar manner, we tested for contingency in the evolution between egg dummy presence/absence and risk of sperm competition. Again, the three-level sperm competition rank was dichotomized by combining the moderate and high risk of sperm competition categories into a single category.

For all BayesTraits analyses, we used phylogenies from the MrBayes phylogenetic inference; hence, branch lengths are in number of expected substitutions. However, we repeated the analyses after transforming branch lengths to reflect time using nonparametric rate smoothing in the package ape (Paradis *et al.*, 2004) in R

(R Development Core Team, 2005) and results do not change (results not shown).

Results

Contingency analysis of egg dummies and parental care

The analysis included a total of 154 species: 69 (45%) species were substrate guarding without egg dummies, 42 (27%) were mouthbrooding with egg dummies and 43 (28%) were mouthbrooding without egg dummies (Fig. 1). We found strong support in favour of a model of dependent evolution of mouthbrooding and egg dummies as compared to the model of independent evolution (log-BayesFactor 15.9). In addition, in the dependent model, the Markov chain never visited an independent model out of the 10 000 iterations in our sample, which provides further strong support for correlated evolution.

The estimates of the transition parameters (Fig. 2, see also Fig. S3 for posterior distributions of the rate coefficients) indicated that evolutionary transitions between mouthbrooding without egg dummies to mouthbrooding with egg dummies were crucial to explain the evolution of these two traits. This is supported by the fact that these transitions were set to a value of zero (i.e. it does not occur; Pagel & Meade, 2006) in a minority of the models visited by the Markov chain ($Z = 0.021$ and < 0.001 respectively; Fig. 2; Gonzalez-Voyer *et al.*, 2008). This should be contrasted to the

transition between substrate guarding without egg dummies and substrate guarding with egg dummies, which is highly unlikely ($Z = 0.957$; Fig. 2). The state combination substrate guarding with egg dummies does not exist in any extant species; hence, the transition rates leading to this state were unlikely ($Z > 0.400$; Fig. 2), whereas transition rates leading away from this state were high ($Z < 0.080$) allowing the model to quickly move out of this trait combination (Fig. 2; Pagel & Meade, 2006). The trait combination substrate guarding without egg dummies was reached directly from mouthbrooding without egg dummies, through a loss of mouthbrooding (Fig. 2), which was the only evolutionary pathway towards this state supported by the model. Furthermore, substrate guarding without egg dummies appeared to be an evolutionary dead-end because transitions leading away from this state either to mouthbrooding without egg dummies or back to substrate guarding with egg dummies were highly unlikely ($Z = 0.943$ and 0.957 , respectively; Fig. 2).

Correlated evolution of egg dummies and precopulatory sexual selection

None of the species in the low intensity of sexual selection category had egg dummies, whereas four of nine (44%) and 24 of 33 (73%) had egg dummies in the moderate and high intensity of sexual selection categories, respectively (Fig. 1). We found that the relative number of species with egg dummies was significantly higher in species with high ($\beta = 1.04$, $CI = 0.21$ – 2.11 ,

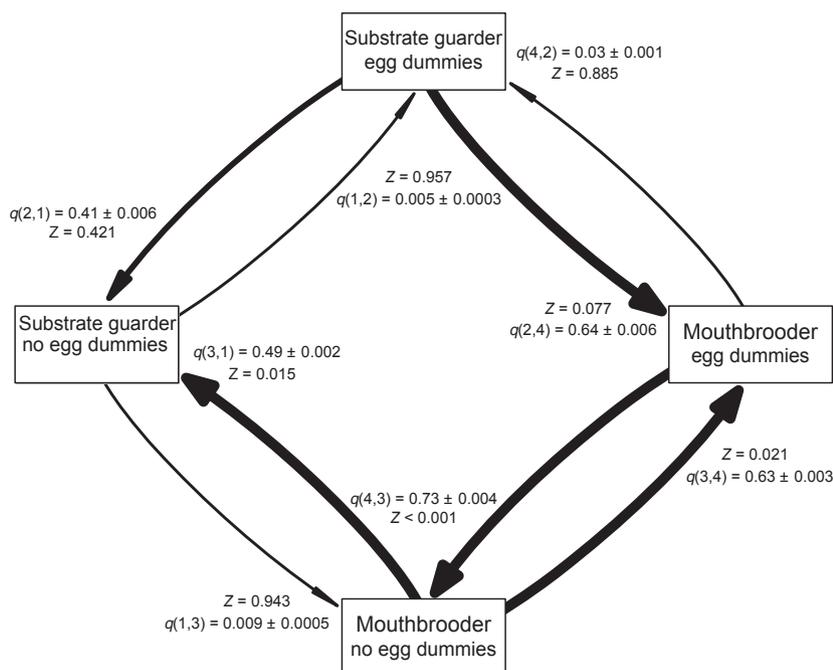


Fig. 2 Flow chart showing the transition rates between the four possible combinations of form of parental care and egg dummy presence/absence. The values associated with the respective transitions are the proportion of iterations in which that transition was set to 0 (Z-value) and the mean \pm SE for the transition parameters (q). Arrow thickness relates to the likelihood of the transitions with thicker arrows representing more likely transitions.

$P = 0.021$) but not moderate ($\beta = 0.81$, $CI = -0.58-2.00$, $P = 0.310$) intensity of sexual selection compared with species with low intensity of sexual selection.

Correlated evolution of egg dummies and post-copulatory sexual selection

Only a single species of 26 in the low risk of sperm competition category had egg dummies (4%), whereas 19 of 27 (70%) and eight of nine (89%) had egg dummies in the moderate and high risk of sperm competition categories, respectively (Fig. 1). We also found that the relative number of species with egg dummies was significantly higher in species with high ($\beta = 1.13$, $CI = 0.02-2.54$, $P = 0.043$) and moderate ($\beta = 1.01$, $CI = 0.17-1.96$, $P = 0.020$) risk of sperm competition compared with species with low risk of sperm competition.

Contingency analysis of egg dummies and precopulatory sexual selection

The analysis included a total of 62 species: 20 (32%) species had low intensity of sexual selection without egg dummies, 14 (23%) species had high intensity of sexual selection without egg dummies, no species presented low intensity of sexual selection with egg dummies, and 28 (45%) had high intensity of sexual selection and egg dummies (Fig. 1). We found strong support in favour of a model of dependent evolution as compared to the model of independent evolution (log-BayesFactor 12.6). In addition, in the dependent model, the Markov chain never visited an independent model out of the 10 000 iterations in our sample, which provides additional strong support for correlated evolution.

Transition parameter estimates (see Fig. S4 for posterior distributions of the rate coefficients) indicated that the evolution of egg dummies was more likely to occur in species with high intensity of sexual selection (evolution of egg dummies in combination with low intensity of sexual selection: $Z = 0.977$ compared with $Z = 0.001$ in combination with high intensity of sexual selection; Fig. 3a). The trait combination low intensity of sexual selection and presence of egg dummies does not exist in any extant species. This is supported by the low likelihood of transitions moving to this state ($Z = 0.977$ for transition from low intensity of sexual selection without egg dummies and $Z = 0.976$ for transitions from high intensity of sexual selection with egg dummies; Fig. 3a).

Contingency analysis of egg dummies and post-copulatory sexual selection

The analysis included a total of 62 species: 25 (40%) species had low risk of sperm competition without egg dummies, nine (15%) species had high risk of sperm competition without egg dummies, one (2%)

species had low risk of sperm competition with egg dummies, and 27 (44%) had high risk of sperm competition with egg dummies (Fig. 1). We found strong support in favour of a model of dependent evolution as compared to the model of independent evolution (log-BayesFactor 10.8). In addition, in the dependent model, the Markov chain never visited an independent model out of the 10 000 iterations in our sample, which provides additional strong support for correlated evolution.

Transition parameter estimates (see Fig. S5 for posterior distributions of the rate coefficients) indicated that the evolution of egg dummies was more likely to occur in species with high risk of sperm competition (evolution of egg dummies in combination with low risk of sperm competition: $Z = 0.851$ compared with $Z = 0.001$ in combination with high risk of sperm competition; Fig. 3b). The trait combination low risk of sperm competition and presence of egg dummies exists only in one extant species (*T. moorii*). This together with the high likelihood of transitions moving away from this state ($Z = 0.062$ for transition to low risk of sperm competition without egg dummies and $Z = 0.070$ for transitions to high risk of sperm competition with egg dummies; Fig. 3b) suggests that this is an unstable state combination.

Discussion

In agreement with previous studies (Salzburger *et al.*, 2005, 2007; Egger *et al.*, 2011), our formal comparative analysis provided additional evidence for the evolution of egg dummies through sensory exploitation across Tanganyikan cichlids, in that egg dummy evolution was contingent on the presence of mouthbrooding. Even more interestingly, we found that egg dummies were more likely to occur in species with high intensity of pre- and post-copulatory sexual selection. In fact, the evolution of egg dummies was contingent on both an increased intensity of precopulatory sexual selection and risk of sperm competition.

Egg dummies and parental care

We found strong support for coevolution between mouthbrooding and the presence of egg dummies. Moreover, the contingency analysis supported the evolution of egg dummies as a secondary response to mouthbrooding (Fig. 2), and this result in turn provides support for the sensory exploitation hypothesis regarding the evolution of egg dummies in this group (see also Salzburger *et al.*, 2005; Egger *et al.*, 2006; Tobler, 2006). It is still possible that mouthbrooding *per se* was not the pre-existing bias that triggered the evolution of egg dummies. Egger *et al.* (2011) suggested that ingestion of carotenoid-rich food, perhaps in the form of eggs (con- or heterospecific), instead was the trigger for

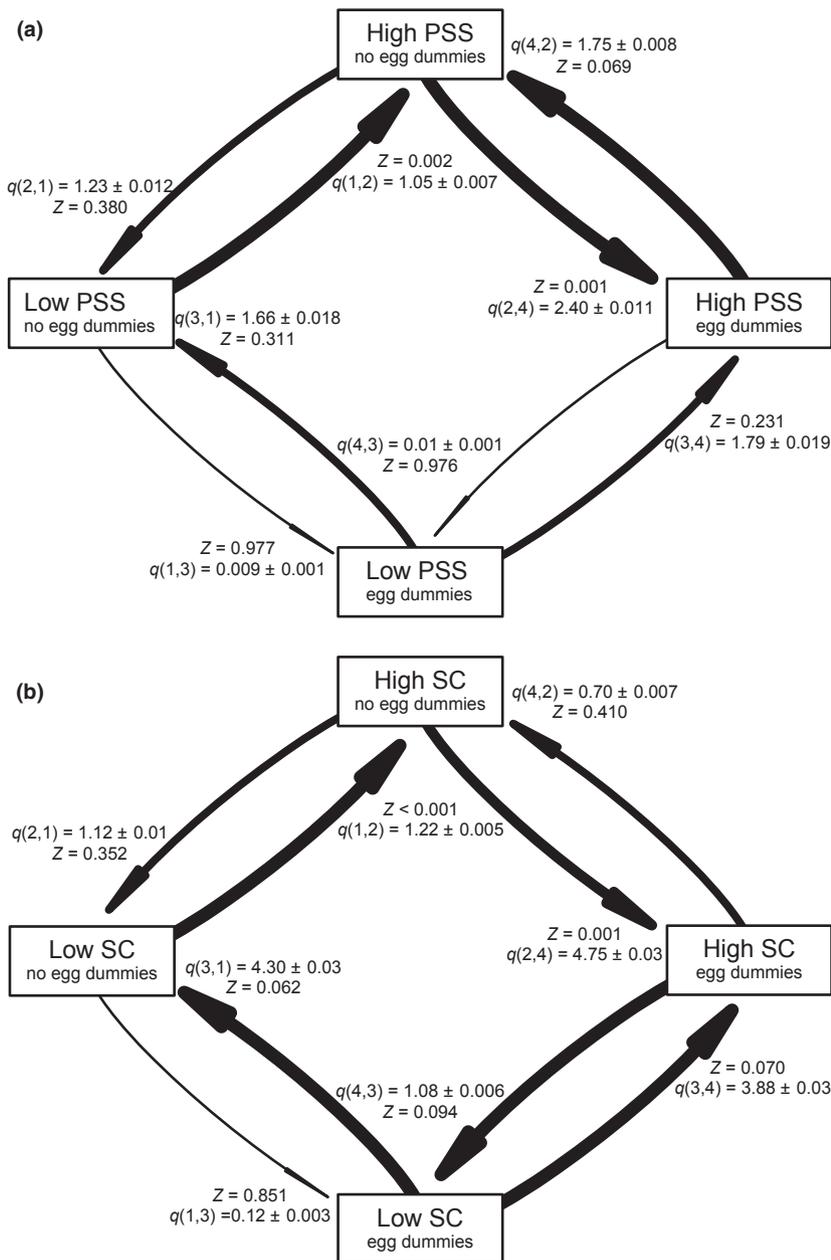


Fig. 3 Flow charts showing the transition rates between the four possible combinations of intensity of precopulatory sexual selection (PSS) and egg dummy presence/absence (a), risk of sperm competition (SC) and egg dummy presence/absence (b). The values associated with the respective transitions are the proportion of iterations in which that transition was set to 0 (Z-value) and the mean \pm SE for the transition parameters (q). Arrow thickness relates to the likelihood of the transitions with thicker arrows representing more likely transitions.

the evolution of egg dummies. They based this idea on the fact that egg-stealing has been observed in cichlids in all the Great Lakes of East Africa (e.g. Nshombo, 1991; Haesler *et al.*, 2009) and that several substrate guarding species also showed a preference for egg dummy-coloured items (Egger *et al.*, 2011). Hence, assuming that substrate guarding preceded mouthbrooding, the preference for egg dummy-like items is likely to have preceded mouthbrooding behaviour (given that substrate guarders also show a preference) and thus that the behaviour of picking up the eggs for brooding might not be the initial bias that triggered the evolution of egg dummies. However, based on our sample

of lake-dwelling Tanganyikan cichlids, mouthbrooding preceded substrate guarding, and an alternative explanation for the preference seen in substrate guarding species without egg dummies is therefore that it is maintained due to benefits in other contexts such as in foraging.

Egg dummies, pre- and post-copulatory sexual selection

Although tests of sexual selection for egg dummies in the form of, for example, mate choice have given mixed results when tested at the species level (e.g. Hert, 1989,

1991; Couldridge & Alexander, 2001; Couldridge, 2002; Henning & Meyer, 2012), we found strong support for an increase in sexual selection being associated with the presence of egg dummies in our large-scale analyses. Is this pattern caused by selection at the premating stage through mate choice or at the post-mating stage through sperm competition or through a combination of both these potential selection pressures? Advantages associated with polyandry may select for multiple mating in females (Birkhead *et al.*, 1987; Keller & Reeve, 1995; Yasui, 2001). Sperm competition is the result regardless of whether polyandry is due to females actively choosing to mate with several males or whether sneakers or predators impose multiple matings (Taborsky, 1994). Irrespective of whether females have the ability to affect the success of different sperm that have been inhaled in the mouth (e.g. Eberhard, 1996; Telford & Jennions, 1998) or whether fertilization success is solely determined by the quality of the respective males' sperm, the latency between the sperm transfer of different males may affect the reproductive success (Taborsky, 1994). We showed here that egg dummy presence increased with risk of sperm competition, and our contingency analysis indicated that high risk of sperm competition favours the evolution of egg dummies. But how do egg dummies help males to increase their success in sperm competition? The location of the egg dummies on the anal fin may ensure that the female's mouth comes close to the genital opening possibly increasing the quantity of inhaled sperm through the nibbling behaviour seen in many species (e.g. Wickler, 1962; Mrowka, 1987a). An alternative but not necessarily contradictory explanation is that the egg dummies could serve to maintain the female close to the male for longer during or following sperm transfer, thereby increasing the reproductive advantage of that particular male by prolonging the time until other males can deposit any sperm.

Our precopulatory sexual selection and sperm competition ranks were strongly correlated, and we can therefore not tease apart which, if any, factor is the key driver in the evolution of egg dummies. For instance, in the moderate risk of sperm competition category, all species were lekking (Table S2). In a lekking mating system, there may be increased premating sexual selection both through female choice and increased male-male competition, in addition to sperm competition. Apart from acting in direct mate choice, the egg-dummies may also function solely by attracting the attention of the female from further away (Hert, 1989). Although we cannot directly tease apart the relative influence of sperm competition and other types of sexual selection, significantly more species with sneaking males had egg dummies. This lends support to our hypothesis that sperm competition may be an important factor in the maintenance of egg dummies in Lake Tanganyika cichlids.

Egg dummies are one of the proposed key innovations responsible for the remarkable species richness in the most species-rich group of cichlids, the Haplochromini (Oppenheimer, 1970; Goldschmidt & de Visser, 1990; Salzburger *et al.*, 2005). Better understanding of both their evolution and present function is an important step towards increasing our understanding of how speciation may have occurred in the haplochromines and other East African cichlids. Variation in abiotic factors such as ambient light and background colour and complexity may affect how well the egg dummies are perceived by the females and thus how well they function as a visual signal (Endler, 1983, 1991). If the egg dummies function as a premating sexual signal or to increase a male's fertilization success in the face of sperm competition, or both, there may be strong selection on the egg dummies to maximize transmission to the females' sensory system, which sets the stage for speciation through sensory drive (e.g. Seehausen *et al.*, 2008). This is true for any signal, regardless of whether it acts in pre- or post-copulatory sexual selection, and their relative importance may vary between species but it is possible that a benefit in sperm competition may be under especially strong selection as it acts in the very final stages of reproduction.

In conclusion, we show that egg dummy evolution, including all possible variants of egg dummies, is contingent on mouthbrooding supporting evolution through sensory exploitation. More importantly, egg dummy presence increased with and was also contingent on the intensity of precopulatory sexual selection as well as the risk of sperm competition. This suggests that in addition to mouthbrooding, these factors may have been important factors in the evolution and maintenance of egg dummies during the Tanganyikan cichlid adaptive radiation.

Acknowledgments

This study was supported by a Swedish Research Council grant to NK. MA was supported by travel grants from the Foundation for Zoological Research (Uppsala University), Helge Ax:son Johnson's Foundation and Sederholms. AG-V was funded by a Juan de la Cierva post-doctoral fellowship from the Spanish Ministerio de Ciencia e Innovación and by a JAE-Doc post-doctoral fellowship from the Consejo Superior de Investigaciones Científicas. We are especially grateful to Erwin Schraml for help with our egg dummy database, to Heinz Büscher for help with scoring the species for sexual dichromatism and to Masanori Kohda, Tetsumi Takahashi, Satoshi Awata, Tetsuo Kuwamura and Tomoki Sunobe for helping us with the data on mating behaviour. Special thanks also to Masahito Tsuboi and Alexander Kotschal for helping us with collection and verifying the data and to Simone Immler and Gavin Thomas for helpful comments on the manuscript.

References

- Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton.
- Balon, E.K. 1977. Early ontogeny of *Labeotropheus* Ahl, 1927 (Mbuna, Cichlidae, Lake Malawi), with a discussion on advanced protective styles in fish reproduction and development. *Env. Biol. Fish.* **2**: 147–176.
- Balshine, S., Leach, B.J., Neat, F., Werner, N.Y. & Montgomerie, R. 2001. Sperm size of African cichlids in relation to sperm competition. *Behav. Ecol.* **12**: 726–731.
- Basolo, A.L. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* **250**: 808–810.
- Birkhead, T.R. & Møller, A.P. 1998. *Sperm Competition and Sexual Selection*. Academic Press, London.
- Birkhead, T.R., Atkin, L. & Møller, A.P. 1987. Copulation behaviour of birds. *Behaviour* **101**: 101–138.
- Chakrabarty, P. 2005. Testing conjectures about morphological diversity in cichlids of Lakes Malawi and Tanganyika. *Copeia* **2**: 359–373.
- Coeckelberghs, V. 1976. Contribution to the ethology of *Limnotilapia dardennesi* (Boulenger, 1899) (Pisces, Cichlidae). I. Description of the elementary actions and the patterns of markings. *Behav. Process.* **1**: 105–124.
- Couldridge, V.C.K. 2002. Experimental manipulation of male eggspots demonstrates female preference for one large spot in *Pseudotropheus lombardoi*. *J. Fish Biol.* **60**: 726–730.
- Couldridge, V.C.K. & Alexander, G.J. 2001. Does the time spent near a male predict female mate choice in a Malawian cichlid? *J. Fish Biol.* **59**: 667–672.
- Day, J.J., Santini, S. & Garcia-Moreno, J. 2007. Phylogenetic relationships of the Lake Tanganyika cichlid tribe Lamprologini: the story from mitochondrial DNA. *Mol. Phylogenet. Evol.* **45**: 629–642.
- Duftner, N., Koblmüller, S. & Sturmbauer, C. 2005. Evolutionary relationships of the Limnochromini, a tribe of benthic deepwater cichlid fish endemic to Lake Tanganyika, East Africa. *J. Mol. Evol.* **60**: 277–289.
- Eberhard, W.G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton.
- Egger, B., Obermüller, B., Phiri, H., Sturmbauer, C. & Sefc, K.M. 2006. Monogamy in the maternally mouthbrooding Lake Tanganyika cichlid fish *Tropheus moorii*. *Proc. R. Soc. Lond. B* **273**: 1797–1802.
- Egger, B., Klafinger, Y., Theis, A. & Salzburger, W. 2011. A sensory bias has triggered the evolution of egg-spots in cichlid fishes. *PLoS ONE* **6**: e25601.
- Emlen, S.T. & Oring, L.W. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* **197**: 215–223.
- Endler, J.A. 1983. Natural and sexual selection on color patterns in poeciliid fishes. *Env. Biol. Fish.* **9**: 173–190.
- Endler, J.A. 1991. Variation in the appearance of guppy patterns to guppies and their predators under different visual conditions. *Vision. Res.* **31**: 587–608.
- Farias, I.P., Ortí, G. & Meyer, A. 2000. Total evidence: molecules, morphology, and the phylogenetics of cichlid fishes. *Mol. Dev. Evol.* **288**: 76–92.
- Fernald, R.D. & Hirata, N.R. 1977. Field study of *Haplochromis burtoni*: Quantitative behavioural observations. *Anim. Behav.* **25**: 964–975.
- Fitzpatrick, J.L., Montgomerie, R., Desjardins, J.K., Stiver, K.A., Kolm, N. & Balshine, S. 2009. Female promiscuity promotes the evolution of faster sperm in cichlid fishes. *Proc. Natl. Acad. Sci. USA* **106**: 1128–1132.
- Fryer, G. & Iles, T.D. 1972. *The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution*. Oliver & Boyd, Edinburgh.
- Garcia, C.M. & Ramirez, E. 2005. Evidence that sensory traps can evolve into honest signals. *Nature* **434**: 501–505.
- Genner, M.J., Seehausen, O., Lunt, D.H., Joyce, D.A., Shaw, P.W., Carvalho, G.R. et al. 2007. Age of cichlids: new dates for ancient lake fish radiations. *Mol. Biol. Evol.* **24**: 1269–1282.
- Goldschmidt, T. 1991. Egg mimics in Haplochromine cichlids (Pisces, Perciformes) from Lake Victoria. *Ethology* **88**: 177–190.
- Goldschmidt, T. & de Visser, J. 1990. On the possible role of egg mimics in speciation. *Acta. Biotheor.* **38**: 125–134.
- Gonzalez-Voyer, A. & Kolm, N. 2011. Rates of phenotypic evolution of ecological characters and sexual traits during the Tanganyikan cichlid adaptive radiation. *J. Evol. Biol.* **24**: 2378–2388.
- Gonzalez-Voyer, A., Fitzpatrick, J.L. & Kolm, N. 2008. Sexual selection determines parental care patterns in cichlid fishes. *Evolution* **62**: 2015–2026.
- Guindon, S. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* **52**: 696–704.
- Hadfield, J.D. 2010. MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**: 1–22.
- Hadfield, J.D. & Nakagawa, S. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies, and multi-trait models for continuous and categorical characters. *J. Evol. Biol.* **23**: 494–508.
- Haesler, M.P., Lindeyer, C.M. & Taborsky, M. 2009. Reproductive parasitism: male and female responses to conspecific and heterospecific intrusions at spawning in a mouthbrooding cichlid *Ophthalmotilapia ventralis*. *J. Fish Biol.* **75**: 1845–1856.
- Haesler, M.P., Lindeyer, C.M., Otti, O., Bonfils, D., Heg, D. & Taborsky, M. 2011. Female mouthbrooders in control of pre- and postmating sexual selection. *Behav. Ecol.* **22**: 1033–1041.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**: 95–98.
- Henning, F. & Meyer, A. 2012. Eggspot number and sexual selection in the cichlid fish *Astatotilapia burtoni*. *PLoS ONE* **7**: e43695.
- Hert, E. 1989. The function of egg-spots in an African mouth-brooding cichlid fish. *Anim. Behav.* **37**: 726–732.
- Hert, E. 1991. Female choice based on egg-spots in *Pseudotropheus aurora* Burgess 1976, a rock-dwelling cichlid of Lake Malawi, Africa. *J. Fish Biol.* **38**: 951–953.
- Heule, C. & Salzburger, W. 2011. The ontogenetic development of egg-spots in the haplochromine cichlid fish *Astatotilapia burtoni*. *J. Fish Biol.* **78**: 1588–1593.
- Höglund, J. & Alatalo, R.V. 1995. *Leks*. Princeton University Press, Princeton.
- Immler, S. & Taborsky, M. 2009. Sequential polyandry affords post-mating sexual selection in the mouths of cichlid females. *Behav. Ecol. Sociobiol.* **63**: 1219–1230.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**: 3059–3066.

- Keller, L. & Reeve, H.K. 1995. Why do females mate with multiple males? The sexually selected sperm hypothesis. *Adv. Study Behav.* **24**: 291–315.
- Kellogg, K.A., Markert, J.A., Stauffer, J.R. Jr & Kocher, T.D. 1995. Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi. *Proc. R. Soc. Lond. B* **260**: 79–84.
- Kellogg, K.A., Markert, J.A., Stauffer, J.R. Jr & Kocher, T.D. 1998. Intraspecific brood mixing and reduced polyandry in a maternal mouth-brooding cichlid. *Behav. Ecol.* **9**: 309–312.
- Koblmüller, S., Duftner, N., Katongo, C., Phiri, H. & Sturmbauer, C. 2005. Ancient divergence in bathypelagic Lake Tanganyika deepwater cichlids: mitochondrial phylogeny of the tribe Bathybatiini. *J. Mol. Evol.* **60**: 297–314.
- Koblmüller, S., Sefc, K.M. & Sturmbauer, C. 2008. The Lake Tanganyika cichlid species assemblage: recent advances in molecular phylogenetics. *Hydrobiologia* **615**: 5–20.
- Kocher, T.D. 2004. Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5**: 288–298.
- Kocher, T.D., Conroy, J.A., McKaye, K.R., Stauffer, J.R. & Lockwood, S.F. 1995. Evolution of NADH dehydrogenase subunit 2 in East African cichlid fish. *Mol. Phylogenet. Evol.* **4**: 420–432.
- Kuwamura, T. 1986. Parental care and mating systems of cichlid fishes in Lake Tanganyika: a preliminary field survey. *J. Ethol.* **4**: 129–146.
- Kuwamura, T. 1987. Male mating territory and sneaking in a maternal mouthbrooder, *Pseudosimochromis curvifrons* (Pisces; Cichlidae). *J. Ethol.* **5**: 203–206.
- Kuwamura, T. 1997. Parental care. In: *Fish Communities in Lake Tanganyika* (H. Kawanabe, M. Hori, M. Nagoshi, eds), pp. 59–86. Kyoto University Press, Kyoto.
- Lehtonen, T.K. & Meyer, A. 2011. Heritability and adaptive significance of the number of egg dummies in the cichlid fish *Astatotilapia burtoni*. *Proc. R. Soc. Lond. B* **278**: 2318–2324.
- Miller, M.A., Holder, M.T., Vos, R., Midford, P.E., Liebowitz, T., Chan, L. *et al.* 2009. The CIPRES Portals. CIPRES. 2009-08-04. URL http://www.phylo.org/sub_sections/portal.
- Møller, A.P. & Ninni, P. 1998. Sperm competition and sexual selection: a meta-analysis of paternity in birds. *Behav. Ecol. Sociobiol.* **43**: 345–358.
- Mrowka, W. 1987a. Oral fertilisation in a mouthbrooding cichlid fish. *Ethology* **74**: 293–296.
- Mrowka, W. 1987b. Egg stealing in a mouthbrooding cichlid fish. *Anim. Behav.* **35**: 923–925.
- Mrowka, W. 1987c. Filial cannibalism and reproductive success in the maternal mouthbrooding cichlid fish *Pseudocrenilabrus multicolor*. *Behav. Ecol. Sociobiol.* **21**: 257–265.
- Nishida, M. 1991. Lake Tanganyika as an evolutionary reservoir of old lineages of East African cichlid fishes: inferences from allozyme data. *Experientia* **47**: 974–979.
- Nshombo, M. 1991. Occasional egg-eating by the scale-eater *Plecodus straeleni* (Cichlidae) of Lake Tanganyika. *Env. Biol. Fish.* **31**: 207–212.
- Ochi, H. 1993. Mate monopolization by a dominant male in a multi-male social group of a mouthbrooding cichlid, *Ctenochromis horei*. *Jpn. J. Ichthyol.* **40**: 209–218.
- Oppenheimer, J.R. 1970. Mouthbreeding in fishes. *Anim. Behav.* **18**: 493–503.
- Pagel, M. & Meade, A. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am. Nat.* **167**: 808–825.
- Pagel, M., Meade, A. & Barker, D. 2004. Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* **53**: 673–684.
- Paradis, E., Claude, J. & Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.
- Parker, A. & Kornfield, I. 1996. Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Env. Biol. Fish.* **47**: 345–352.
- Poll, M. 1986. Classification des cichlidae du lac Tanganyika. Tribus, genres et espèces. *Mem. Acad. R. Belg. Cl. Sci.* **45**: 1–163.
- Posada, D. 2008. jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**: 1253–1256.
- Proctor, H.C. 1991. Courtship in the water mite *Neumania papillator*: males capitalize on female adaptations for predation. *Anim. Behav.* **42**: 589–598.
- R Development Core Team 2005. *R: A Language and Environment for Statistical Computing*. R foundation for statistical computing, Vienna, Austria.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Ryan, M.J. & Rand, A. 1990. The sensory basis of sexual selection for complex calls in the túngara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). *Evolution* **44**: 305–314.
- Salzburger, W. 2009. The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Mol. Ecol.* **18**: 169–185.
- Salzburger, W. & Meyer, A. 2004. The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften* **91**: 277–290.
- Salzburger, W., Meyer, A., Baric, S., Verheyen, E. & Sturmbauer, C. 2002. Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African Haplochromine cichlid fish faunas. *Syst. Biol.* **51**: 113–135.
- Salzburger, W., Mack, T., Verheyen, E. & Meyer, A. 2005. Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evol. Biol.* **5**: 17.
- Salzburger, W., Braach, I. & Meyer, A. 2007. Adaptive sequence evolution in a colour gene involved in the formation of the characteristic egg dummies of male haplochromine cichlid fishes. *BMC Biol.* **5**: 51.
- Seehausen, O. 2006. African cichlid fish: a model system in adaptive radiation research. *Proc. R. Soc. Lond. B* **273**: 1987–1998.
- Seehausen, O., Terai, Y., Magalhaes, I.S., Carleton, K.L., Mrosso, H.D.J., Miyagi, R. *et al.* 2008. Speciation through sensory drive in cichlid fish. *Nature* **455**: 620–626.
- Sefc, K.M. 2011. Mating and parental care in Lake Tanganyika's cichlids. *Int. J. Evol. Biol.* **2011**: Article ID 470875.
- Sefc, K.M., Hermann, C.M. & Koblmüller, S. 2009. Mating system variability in a mouthbrooding cichlid fish from a tropical lake. *Mol. Ecol.* **18**: 3508–3517.
- Snoeks, J. 2000. How well known is the ichthyodiversity in the large East African lakes? *Adv. Ecol. Res.* **31**: 17–38.
- Stamatakis, A. 2006. RAxML-VI-HPC: maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

- Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* **57**: 758–771.
- Sturmbauer, C., Hainz, U., Baric, S., Verheyen, E. & Salzburger, W. 2003. Evolution of the tribe Tropheini from Lake Tanganyika: synchronized explosive speciation producing multiple evolutionary parallelism. *Hydrobiologia* **500**: 51–64.
- Taborsky, M. 1994. Sneakers, satellites and helpers: parasitic and cooperative behaviour in fish reproduction. *Adv. Study Behav.* **23**: 1–100.
- Taborsky, M. 1998. Sperm competition in fish: “bourgeois” males and parasitic spawning. *Trends Ecol. Evol.* **13**: 222–227.
- Takahashi, T. 2003. Systematics of Tanganyikan cichlid fishes (Teleostei: Perciformes). *Ichthyol. Res.* **50**: 367–382.
- Takahashi, T. & Hori, M. 2006. Description of a new Lake Tanganyika cichlid of the genus *Cyprichromis* (Perciformes: Cichlidae) with a note on sexual dimorphism. *J. Fish Biol.* **68**: 174–192.
- Takahashi, T. & Koblmüller, S. 2011. The adaptive radiation of cichlid fish in Lake Tanganyika: a morphological perspective. *Int. J. Evol. Biol.* **2011**: Article ID 470875.
- Takahashi, T., Ochi, H., Kohda, M. & Hori, M. 2011. Invisible pair bonds detected by molecular analyses. *Biol. Lett.* **8**: 355–357.
- Taylor, M.I., Morley, J.I., Rico, C. & Balshine, S. 2003. Evidence of genetic monogamy and female-biased dispersal in the biparental mouthbrooding cichlid *Eretmodus cyanostictus* from Lake Tanganyika. *Mol. Ecol.* **12**: 3173–3177.
- Telford, S.R. & Jennions, M.D. 1998. Establishing cryptic female choice in animals. *Trends Ecol. Evol.* **13**: 216–218.
- Theis, A., Salzburger, W. & Egger, B. 2012. The function of anal fin egg-spots in the cichlid fish *Astatotilapia burtoni*. *PLoS ONE* **7**: e29878.
- Tobler, M. 2006. The eggspots of cichlids: evolution through sensory exploitation? *Z. Fischkunde* **8**: 39–46.
- Turner, G.F., Seehausen, O., Knight, M.E., Allender, C.J. & Robinson, R.L. 2001. How many species of cichlids are there in African lakes? *Mol. Ecol.* **10**: 793–806.
- Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. 2003. Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science* **300**: 325–329.
- Wagner, C.E., Harmon, L.J. & Seehausen, O. 2012. Ecological opportunity and sexual selection together predict adaptive radiation. *Nature* **487**: 366–369.
- West-Eberhard, M.J. 1984. Sexual selection, competitive communication and species-specific signals in insects. In: *Insect Communication* (T. Lewis, ed.), pp. 283–324. Academic Press, New York.
- Wickler, W. 1962. “Egg-dummies” as natural releasers in mouth-breeding cichlids. *Nature* **194**: 1092–1094.
- Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. 2004. AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. URL <http://ceb.fsu.edu/awty>.
- Xia, X. 2001. *Data Analysis in Molecular Biology and Evolution*. Kluwer Academic Publishers, Boston.
- Yasui, Y. 2001. Special submission as a genetic bet-hedging strategy when mate choice criteria are unreliable. *Ecol. Res.* **16**: 605–616.
- Young, K.A., Snoeks, J. & Seehausen, O. 2009. Morphological diversity and the roles of contingency, chance and determinism in African cichlid radiations. *PLoS ONE* **4**: e4740.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Species included in contingency analysis of form of parental care and egg dummies.

Table S2 Data on mating behaviour and sexual dichromatism for mouthbrooders and the precopulatory sexual selection (PSS) and sperm competition (SC) rank.

Table S3 GenBank accession numbers for the sequences included in the phylogenetic analysis including *Heterochromis multidentis* as outgroup.

Figure S1 Observed transitions (blue crosses and line) and transversions (green triangles and line) against a corrected genetic distance for NADH2 including the outgroup *H. multidentis*.

Figure S2 Observed transitions (blue crosses and line) and transversions (green triangles and line) against a corrected genetic distance for cytochrome B including the outgroup *H. multidentis*.

Figure S3 Posterior distributions of the rate coefficients of the model of trait evolution (form of parental care and egg dummy presence/absence) in a model of dependent evolution with a gamma prior.

Figure S4 Posterior distributions of the rate coefficients of the model of trait evolution (intensity of precopulatory sexual selection and egg dummy presence/absence) in a model of dependent evolution with a gamma prior.

Figure S5 Posterior distributions of the rate coefficients of the model of trait evolution (risk of sperm competition and egg dummy presence/absence) in a model of dependent evolution with a gamma prior.

Received 25 April 2013; revised 22 July 2013; accepted 22 July 2013