

Living on the wedge: female control of paternity in a cooperatively polyandrous cichlid

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Theories suggest that, in cooperatively breeding species, female control over paternity and reproductive output may affect male reproductive skew and group stability. Female paternity control may come about through cryptic female choice or female reproductive behaviour, but experimental studies are scarce. Here, we show a new form of female paternity control in a cooperatively polyandrous cichlid fish (*Julidochromis transcriptus*), in which females prefer wedge-shaped nesting sites. Wedge-shaped sites allowed females to manipulate the siring success of the group member males by spawning the clutch at the spot where the large males were just able to enter and fertilize the outer part of the clutch. Small males fertilized the inner part of the clutch, protected from the large aggressive males, leading to low male reproductive skew. Small males provided more brood care than large males. Multiple paternity induced both males to provide brood care and reduced female brood care accordingly. This is, to our knowledge, the first documented case in a species with external fertilization showing female mating behaviour leading to multiple male paternity and increased male brood care as a result.

Keywords: female paternity control; reproductive skew; cooperative polyandry; sexual conflict; brood care; *Julidochromis transcriptus*

1. INTRODUCTION

Cooperative polyandry, where subordinate males may share paternity and brood care with a dominant male, has been well documented in birds (Brown 1987; Emlen 1997; Koenig & Dickinson 2004). Males are usually unrelated and reproduction is often evenly divided among the males ('low reproductive skew', e.g. Burke *et al.* 1989; Jamieson *et al.* 1994; Whittingham *et al.* 1997; Williams 2004). In extended family groups, male offspring help their dominant parents to rear younger siblings in their natal territory and typically reproduction is highly skewed towards dominant males ('high reproductive skew', e.g. Brown 1987; Koenig & Dickinson 2004). Theoretical and empirical studies have focused on the social factors influencing reproductive skew (e.g. relatedness and inbreeding avoidance, Vehrencamp 1983; Johnston 2000; Heg *et al.* 2006). Reproductive skew models assume that either (i) dominants control the degree of reproductive skew (Vehrencamp 1983; Johnston 2000), (ii) subordinates

refrain from reproduction (Reeve & Keller 2001; Koenig & Dickinson 2004), or (iii) skew is determined by a tug-of-war between dominants and subordinates over reproduction (Johnston 2000; Reeve & Keller 2001; Heg *et al.* 2006; Hamilton & Heg 2007; Heg & Hamilton 2008). In all cases, reproductive skew is regarded as being governed by within-sex interactions.

The effect of strategic adjustments by a third party on dominant–subordinate interactions and reproductive skew has been acknowledged in theoretical (Stacey 1982; Cant & Reeve 2002; Hamilton & Heg 2007) and empirical (Burke *et al.* 1989; Davies *et al.* 1996; Whittingham *et al.* 1997; Haydock & Koenig 2002; Li & Brown 2002; Williams 2004) studies, but the effect has rarely been tested experimentally. For example, females (the third party) might control the paternity of the dominant and subordinate group member males by cryptic female choice (e.g. sperm choice, Birkhead & Møller 1993; Eberhard 1996) or reproductive behaviour (e.g. sneaky copulation with subordinate males, Eason & Sherman 1995; Davies *et al.* 1996), which may influence reproductive skew, group stability and male brood care behaviour (Cant & Reeve 2002; Hamilton & Heg 2007). Females may benefit from mating with multiple males because of increased help by their males (Davies *et al.* 1996), inbreeding avoidance (e.g. Pusey & Wolf 1996;

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Koenig *et al.* 1998; Cooney & Bennett 2000; Cockburn *et al.* 2003; Griffin *et al.* 2003) and decreased risk of infanticide (e.g. Heistermann *et al.* 2001; East *et al.* 2003; Wolff & Macdonald 2004).

Recently, cooperative polyandry has been detected in Lake Tanganyika cichlids, *Chalimochromis brichardi* and *Julidochromis* spp. (monogamy and polygynandry are also present, see Awata & Kohda 2004; Awata *et al.* 2005, 2006; M. Kohda, S. Awata, H. Munehara, T. Takeyama, T. Takahashi, K. Watanabe, M. Hori & D. Heg 2004, unpublished data). These cichlids provide excellent model species to test whether, how and with what consequences females may control paternity and thus reproductive skew among the group member males. Polyandrous cichlid females mating with two or more males often enjoy higher reproductive success than monogamous pairs (Awata *et al.* 2005). Field observations indicate that female cichlids might control the paternity of the males by choosing wedge-shaped crevices as spawning sites (M. Kohda, S. Awata, H. Munehara, T. Takeyama, T. Takahashi, K. Watanabe, M. Hori & D. Heg 2004, unpublished data). Small subordinate males (henceforth called 'beta males') can hide from the large dominant males (called 'alpha males') deep inside these wedges and fertilize (part of) the clutches unharassed by the dominant alpha males; thus, females may be able to mate in polyandry. These size differences between group members of *Julidochromis* species are those typically found in nature (i.e. alpha male > female > beta male, Awata & Kohda 2004; Heg & Bachar 2006) and also in other cooperatively breeding cichlids (see Heg *et al.* 2005 for review). The female control hypothesis states that (i) females prefer wedge-shaped nesting sites to share the paternity with multiple males, (ii) females strategically place the eggs in wedges so beta males can fertilize the eggs laid in the inner part and alpha males the eggs in the outer part, and (iii) shared paternity increases male brood care, so females can reduce their brood care.

To test the female control hypothesis, we conducted aquarium experiments using the cooperatively polyandrous cichlid *Julidochromis transcriptus*, in which both alpha and beta males participate in reproduction and jointly take care of broods (Awata *et al.* 2006, 2008). Within cooperative breeding groups, alpha males are larger than the females, and the beta males are smaller than the females (Awata *et al.* 2008). We created breeding pairs (female with alpha or beta male) and trios (female with both males) in various experiments. In the first experiment we tested whether females in pairs or in trios prefer wedge-shaped sites for spawning (experiment 1a, see figure 1a and table S1 in the electronic supplementary material). The hypothesis tested is that females only prefer wedge-shaped nests in trios (i.e. to allow beta males access to spawning and induce beta male care). Then, to verify the significance of using wedge-shaped sites, only an open (non-wedge) nest was given to trios and their nest usage was observed (experiment 1b, see table S1 in the electronic supplementary material). In the second experiment we examined the reproductive behaviour of females in trios, and compared them to females paired only with an alpha male or a beta male (experiment 2, see figure 1b,c and table S2 in the electronic supplementary material).

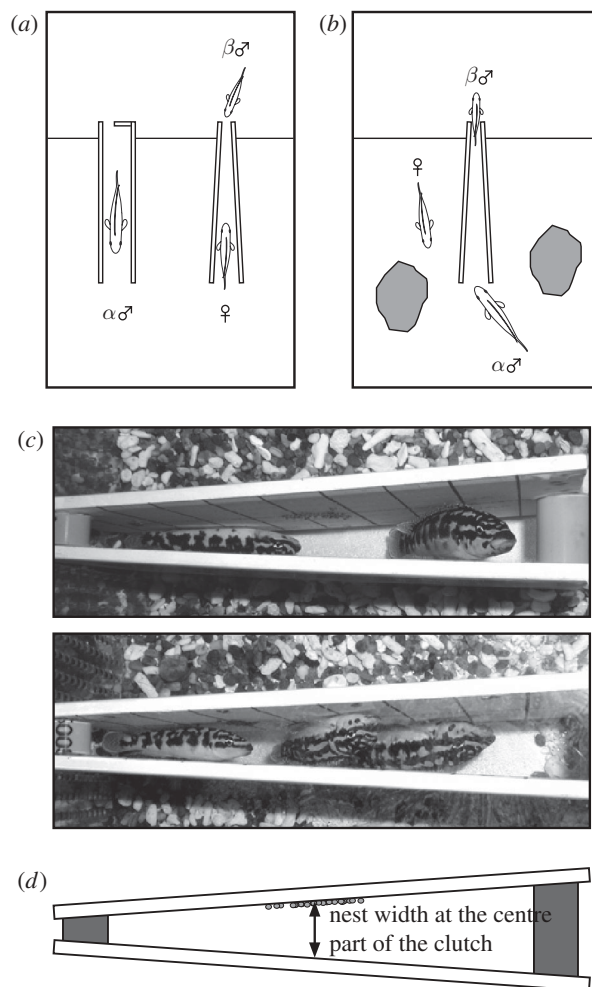


Figure 1. Setup of the two experiments using the cooperatively polyandrous cichlid *Julidochromis transcriptus*. (a) Experiment 1a. Pairs or trios had access to both a wedge-shaped and an open nest. This example shows a trio. Note that the alpha male cannot leave the bottom compartment. In experiment 1b, trios had access to an open nest only. (b) Experiment 2. Pairs or trios had access only to one wedge-shaped nest. This example shows a trio. (c) Photographs of a pair of a female and a large alpha male (above) and three members of a trio (below) in the wedge-shaped nest during experiment 2. Clutches can be seen inside the nests. (d) Schematic of the wedge with a clutch attached. From the photographs, the clutch size (egg number) and the clutch position (midpoint, cm) were determined for each brood. Offspring of trios were raised in isolation after behavioural observations from the inner part and outer part of each clutch separately, and DNA genotyped.

2. MATERIAL AND METHODS

(a) Subject fish and maintenance

The small substrate-brooding cichlid *Julidochromis transcriptus* (max. 90 mm in total length, TL) inhabits shallow rocky shores of Lake Tanganyika. *Julidochromis transcriptus* lives in monogamous pairs or cooperatively polyandrous groups. Occasionally, large males or large females are found to mate with several individuals at different breeding nests (polygyny or 'classical polyandry', Awata *et al.* 2006, 2008). Females lay eggs in rock crevices, and group members jointly care for their offspring (Awata *et al.* 2006). Previous laboratory experiments show that eggs hatch into wrigglers after approximately 4 days, and the offspring absorb their yolk sacs and become free-swimming fry at approximately the 8 day wriggler stage

(Awata *et al.* 2006). Note that large alpha males and small beta males are most likely, not two morphs genetically determined (Awata *et al.* 2008), but rather fish of different ages owing to indeterminate growth (e.g. Skubic *et al.* 2004).

Experiments were conducted in a laboratory at Osaka City University, Osaka, Japan. In the experiments, we used fish purchased from several different pet stores and their F₁ descendants raised in our laboratory. The holding conditions are described elsewhere (Awata *et al.* 2006, 2008). The fish in the holding tanks were sexed by the shape of genital papilla under a binocular microscope and measured for TL (to the nearest 0.1 mm) before being used in experiments.

(b) *Experimental tanks*

Experimental tanks measured 45 × 30 × 30 cm³ (length, breadth, height: 40 l) and contained approximately 2 cm of gravel mixed with coral sand. Two types of artificial nests were constructed: a wedge-shaped nest and an open nest (figure 1*a,b*). These nests were made from two slate tiles (10 × 20 × 0.5 cm³) with PVC tubes cut in small pieces for props. The wedge-shaped nests (figure 1*c,d*) were wide at one end (25 mm) and narrow (9 mm) at the other end. The open nests were wide throughout (20 mm, figure 1*a*). Each tank was divided into two compartments by a slotted transparent Plexiglas partition that had one or two slits, into which the nests were fitted (figure 1*a–c*), so that only beta males and smaller females (<7 cm TL) could swim through both compartments via the nest(s). Fish were fed twice daily on commercial Tetramin flakes.

(c) *Experiment 1*

Polyandrous trios ($n = 10$) and monogamous pairs with alpha males ($n = 9$) or with beta males ($n = 9$) were made (alpha males larger than females, females larger than beta males, see table S1 in the electronic supplementary material). Size differences between alpha males and trio females, between trio females and beta males, and within pairs were set around 10–15 mm, as generally observed in the field (Awata & Kohda 2004; Awata *et al.* 2005). In experiment 1*a*, a wedge-shaped and an open nest were simultaneously available to either a pair or a trio (figure 1*a*). To avoid initial male–male harassment in trios, the alpha male and the female were released in the nest compartment, and the beta male in the non-nest compartment (day 0). In pairs, the female and the male were released in the nest compartment (day 0). Fifteen-minute focal observations were conducted daily from day 1 onwards for 5 days, in which we recorded for each individual the percentage of the observation time spent in each nest and the number of attacks performed against each co-inhabitant of the tank. The mean percentage time per individual per nest type (wedge-shaped or open) and mean number of attacks per 15 min, were used for further analyses (based on five observations per tank). For each female we determined whether she spawned her first clutch in the wedge-shaped or open nest, and this was used as an indicator of her nest type preference.

In experiment 1*b*, we conducted an additional experiment to verify that beta males are competitively excluded from the open nests by the alpha males. The procedure was identical to experiment 1*a*, but only polyandrous trios ($n = 10$) were used (see table S1 in the electronic supplementary material), and they were given access to open nests. Again, the mean percentage time per individual spent in the open nest, and the mean number of attacks per 15 min, were used for further analyses (based on three or four observations per tank, days 2 to 4).

(d) *Experiment 2*

In experiment 2, we provided only one wedge-shaped nest site for breeding to a trio ($n = 10$), or a pair with an alpha male ($n = 7$), or a pair with a beta male ($n = 9$; see table S2 in the electronic supplementary material and figure 1*b*). We used unrelated individuals obtained from multiple commercial breeders when establishing polyandrous trio groups, as found in cooperative breeding groups in the wild (Awata *et al.* 2005). From each individual in trios, we preserved a small fin clip taken from the anal fin in 99 per cent ethanol for further parentage analyses (see below).

Trials lasted 94 days on average (\pm s.d. = 34, range = 43–162, $n = 26$; see table S2 in the electronic supplementary material). We checked for newly deposited eggs in the nests every day. Direct brood care by each individual was recorded for 20 min between 10:00 and 15:00 during the first 2 or 3 days after the eggs were laid until hatching (data averaged per individual before further analyses). The focal individuals were allowed to acclimatize to the observer for 5 min before the observation started. We recorded brood care frequencies for each individual (fanning and egg mouthing frequencies summed). Fanning is considered an egg oxygenating behaviour and mouthing is considered an egg/embryo cleaning behaviour (Awata *et al.* 2006).

(e) *Clutches and parentage analyses*

When the clutch was complete, it was photographed using a digital camera (Camedia C-3040, Olympus, Japan), and the photographs were used for counting eggs and determining the position of the eggs (figure 1*c,d*). Each female spawned six times on average (\pm s.d. = 2, range = 2–10, $n = 26$; see table S2 in the electronic supplementary material), and average number of eggs per clutch was 36 (\pm s.d. = 23, range = 2–104, $n = 139$; eggs could not be reliably counted for six broods). On the day of hatching (4 days after spawning), the brood was gently removed from the monogamous parents and the nest replaced. In the case of polyandrous trios, the nests with eggs were removed and replaced with another nest 2 days after spawning. These eggs were processed for parentage analyses. They were incubated artificially in a separate small aquarium with aeration until offspring had grown to 15–30 mm TL (60–100 days of age, 15 broods of three trio groups, offspring were DNA sampled in 25% of the eggs). To increase the sample size per brood, later broods (25 broods of seven trio groups) were collected on the day of hatching (offspring were DNA sampled in 67% of the eggs). Offspring were killed with an overdose of the anaesthetic FA100 (Tanabe Seiyaku Inc., Osaka, Japan) and preserved in 99 per cent ethanol for paternity analyses. In the case that trio females laid eggs widely along the horizontal, the eggs were collected separately for the front and the back part of the clutch.

Parentage analyses were conducted for the trios: (i) all adults and artificially raised offspring were DNA sampled and (ii) paternity was determined from the analysis of four polymorphic microsatellite loci Chb1, Chb2, Pzeb1 and Pzeb3 (Van Oppen *et al.* 1997; Munehara *et al.* 2001; see the electronic supplementary material for details). Of the 512 offspring analysed, 495 (96.7%) were successfully typed, but we failed to extract DNA from 17 offspring samples. Three broods had only one offspring genotyped and were omitted from the analyses, giving a total sample size of 492 offspring. All offspring could be successfully assigned to their father; that is, they had matching alleles for each locus with one male only, and one or more mismatches with the other male.

(f) Statistical analyses

We used the computer software R.2.4.1 throughout (R Development Core Team 2006). Female preferences for staying in wedge-shaped nests and open nests were compared between trios and pairs using non-parametric two-way ANOVAs (Meddis 1984). The paternity, reproductive success and brood care data were analysed using generalized estimating equations (GEEs) to account for repeated measures per trial (i.e. multiple broods collected per trial). The proportion of offspring sired by either alpha or beta males was analysed with a binomial error structure and a weighted logit-link function. The number of offspring sired by either alpha or beta male was analysed with a Poisson error structure and a log-link function. The clutch position was compared between trios and pairs using a GEE with a Gaussian error structure and an identity-link function and with female TL as a covariate.

3. RESULTS

As expected, in experiment 1a, females in pairs did not prefer wedge-shaped nests for shelter (Wilcoxon signed-rank test, time in wedge versus open, $T = 7.5$, $p = 0.20$ in female with alpha male; $T = 22.0$, $p > 0.99$ in female with beta male; figure 2a), and spawning (two binomial tests, $p > 0.99$ in both types of pairs; figure 2b). Neither alpha nor beta males in pairs showed such preferences ($T = 12.0$, $p = 0.25$ in the alpha male; $T = 18.0$, $p > 0.99$ in the beta male; figure 2a). By contrast, in trios, females (percentage of time: $86.9\% \pm 4.4$ s.e.m.) and beta males ($79.4\% \pm 8.1$) stayed in wedge nests much more than in open nests ($T = 0$, $p = 0.002$ in both; figure 2a), whereas alpha males did not show such preferences ($T = 19.0$, $p = 0.43$). Females in trios stayed in wedge nests much longer than pair females with beta males (non-parametric two-way ANOVA, nest shape \times male treatment: $z = 2.90$, $p = 0.004$), but not longer than pair females with alpha males (nest shape \times male treatment, $z = 0.79$, $p = 0.43$; nest shape, $z = 3.52$, $p < 0.001$). There was a non-significant trend suggesting that on the day after release (day 1), trio females were more likely to stay in the wedge nest ($77.3\% \pm 13.0$ s.e.m.) than the trio beta males ($51.2\% \pm 14.1$, Wilcoxon signed-rank test, $T = 8.0$, $p = 0.098$, $n = 10$). Taken together, these results suggest that trio females already prefer wedge nests in the pre-spawning period, but do make visits to the open nest when available. Eventually, all trio females spawned clutches inside the wedge, but not in the open nest (binomial test, $p = 0.002$; figure 2b). The critical tests are that females preferred to spawn in the wedge nest when both alpha and beta males were present compared to when only alpha males (Fisher's exact test, d.f. = 1, $p = 0.033$) or only beta males (d.f. = 1, $p = 0.011$) were present. This suggests that females do not follow a fixed nest preference strategy and also do not follow the beta males or alpha males invariably to their preferred nest site.

In the wedge nests of trios, alpha males reached the average point of 10.2 cm (± 0.9 s.e.m.; or 16.8 mm ± 0.7 s.e.m. in nest width) from the wide end, and females reached significantly deeper inside (17.6 cm ± 0.8 ; or 10.9 mm ± 0.6 ; Wilcoxon signed-rank test, distance $T = 0.0$, $p = 0.002$, $n = 10$, width $T = 0.0$, $p = 0.002$, $n = 10$). Note that all beta males reached the narrow end (figure 1b,c). Alpha males showed aggressive displays

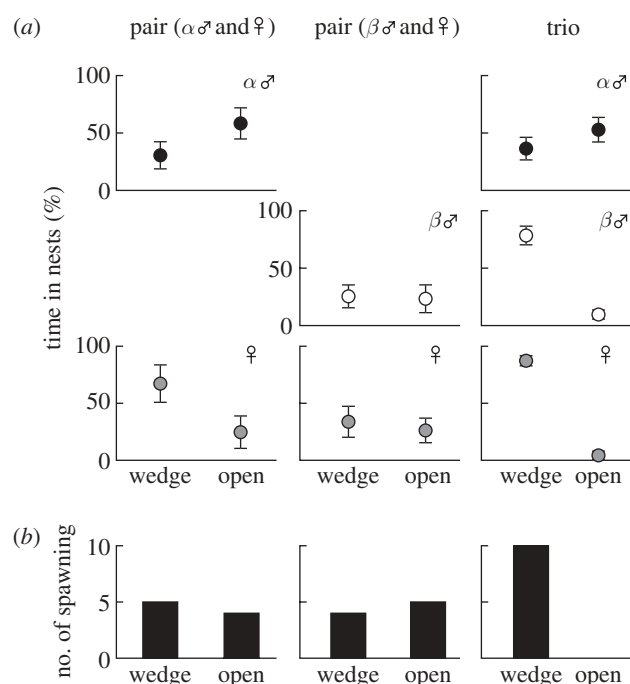


Figure 2. Nest preference and use: wedge nests versus open nests. (a) Percentage of time spent in the wedge-shaped or open nest for alpha males, beta males and females in pairs (both $n = 9$) and trios ($n = 10$). Time outside nests is not shown. Error bars indicate s.e.m. (b) Number of wedge-shaped and open nests females used for their first clutch in pairs (both $n = 9$) and trios ($n = 10$). Data are from experiment 1a.

towards beta males inside the narrower part of wedge (mean frequency \pm s.e.m. per 15 min = 2.3 ± 0.9 , $n = 10$), but could not repel them. In experiment 1b, where only an open nest was given, females ($91.2\% \pm 5.0$, $n = 10$) and alpha males ($78.0\% \pm 7.0$) mostly stayed in the open nest, but beta males ($0.5\% \pm 0.2$) were hardly allowed to enter owing to immediate attacks by the alpha males (4.3 ± 1.1 attacks per 15 min, $n = 10$). Consequently, beta males in trios spent significantly more time in a nest when a wedge-shaped nest was available compared to when only an open nest was available (data as shown above; wedge versus open = 79.4% versus 0.5% , Mann-Whitney U -tests: $U = 0$, $p = 0.0001$). These experiments show that females of *J. transcriptus* in trios prefer to use the wedge-shaped nesting sites, which allow both beta and alpha males to enter the nest site and participate in spawning.

The second prediction is that wedge-shaped nests allowed females to divide the paternity among multiple males. Beta males are expected to fertilize the eggs laid in the inner part of the wedge, alpha males in the outer part of wedge, and thus the paternity distribution will change according to the egg and clutch position in the wedge-shaped sites. Overall, paternity of 39 different clutches in 10 trios was not biased to either alpha (237 offspring, 48.2%) or beta (255 offspring, 51.8%; GEE with log link, Wald $\chi^2_1 = 0.08$, $p = 0.77$) males, indicating low male reproductive skew. However, as predicted, eggs in the frontal, wider part of the clutches were predominantly sired by the alpha males, whereas the beta males sired more offspring in the back, narrower part of the clutches (GEE with weighted logit link, Wald $\chi^2_1 = 5.22$, $p = 0.022$; figure 3a). Similarly, the proportion of offspring sired by beta males

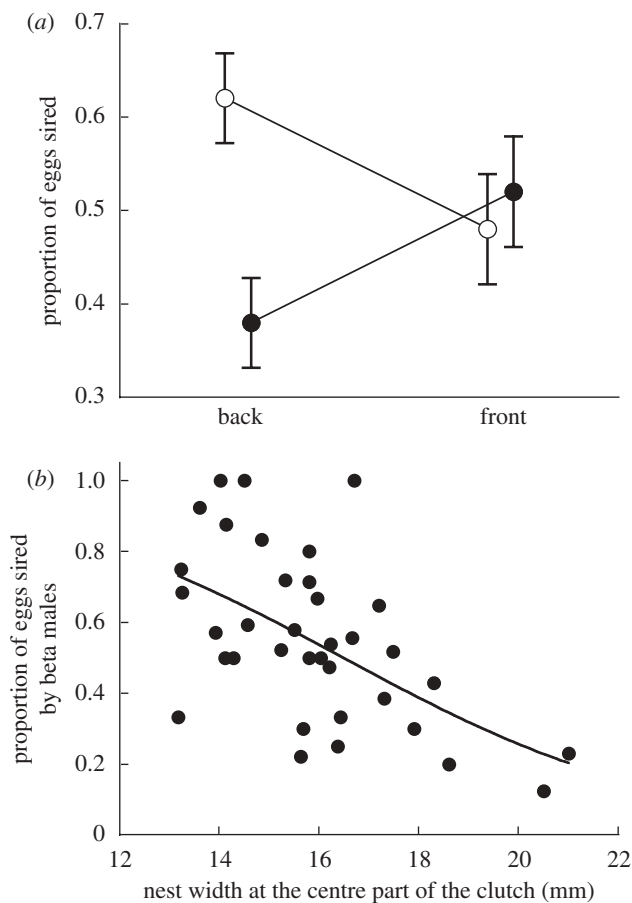


Figure 3. Female control of paternity. (a) Beta males sired more offspring in the inner part of the clutch, whereas alpha and beta males sired similar numbers of offspring in the outer part of the clutch. Offspring ($n = 297$) from 16 different clutches in eight trios. Black circles, sired by alpha males; white circles, sired by beta males. Error bars indicate s.e.m. (b) Beta males were more likely to sire offspring when the clutch was positioned at the narrow part of the wedge. Offspring ($n = 492$) from 39 clutches produced by 10 trios. Data are from experiment 2.

increased when eggs were deposited at the more inner part of the wedge (GEE with weighted logit link, Wald $\chi^2_1 = 57.68$, $p < 0.0001$; figure 3b). Trio females spawned at the more inner part of the wedge than pair females with beta males (GEE with identity link, Wald $\chi^2_1 = 10.60$, $p = 0.001$). Females appear to manipulate the likelihood of both males gaining paternity by choosing the position of the clutch inside the wedge carefully.

The third prediction is that shared paternity increases the brood care performed by the males, and it reduces female brood care. Neither alpha nor beta males showed an increase in brood care behaviour with siring success (alpha males, GEE with weighted logit link, Wald $\chi^2_1 = 0.78$, $p = 0.38$; beta males, Wald $\chi^2_1 = 0.61$, $p = 0.44$, $n = 26$ observations of nine trios). However, females had a lot to gain from inducing beta male brood care: (i) in trios, beta males performed more brood care than alpha males (GEE with weighted logit link, Wald $\chi^2_1 = 11.05$, $p < 0.001$; figure 4a), (ii) beta males in pairs (where they have full paternity) provided more brood care than beta males in trios (Wald $\chi^2_1 = 3.98$, $p = 0.046$; figure 4a), and (iii) alpha males in pairs tended to provide less brood care compared to alpha males in trios (Wald $\chi^2_1 = 2.99$, $p = 0.083$; figure 4a). Thus, total male brood care

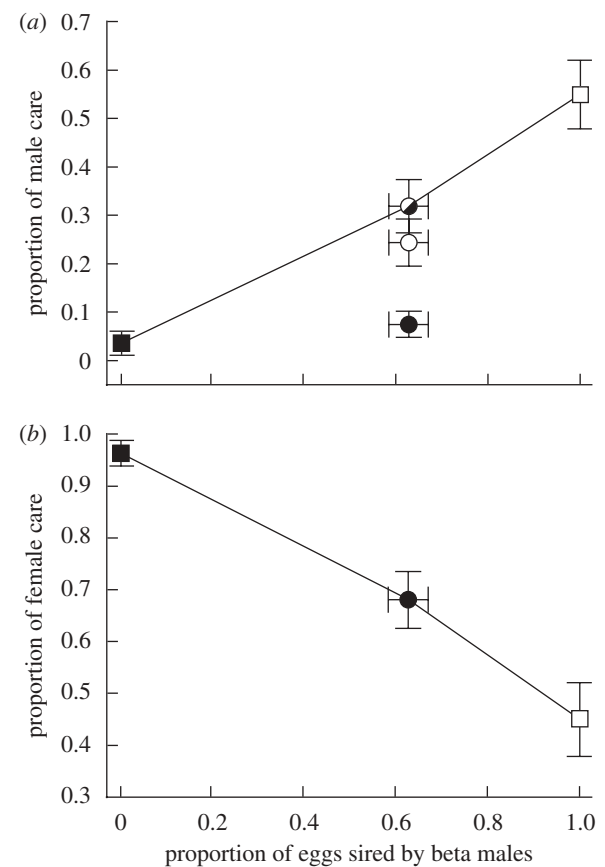


Figure 4. Paternity, mating system and brood care. (a) Beta males provided more brood care than alpha males in all situations (black square, pair of alpha males; black circle, trio of alpha males; white square, pair of beta males; white circle, trio of beta males; black and white circle, total care by trio males). (b) Females reduced their brood care when assisted by a beta male, both in trios and in pairs (black square, pair with alpha male; black circle, trio; white square, pair with beta male). Note the average siring rate of 62 per cent by beta males in trios. Error bars indicate s.e.m. Data are from experiment 2.

in trios was much larger than brood care by alpha males in pairs (Wald $\chi^2_1 = 15.08$, $p < 0.001$), but was not different from brood care by beta males in pairs (Wald $\chi^2_1 = 2.04$, $p = 0.15$; figure 4a). Consequently, females in trios provided less brood care than females paired with only alpha males (Wald $\chi^2_1 = 15.94$, $p < 0.0001$), but was similar to females paired only to a beta male (Wald $\chi^2_1 = 2.33$, $p = 0.13$; figure 4b). We conclude that females may reduce their share in brood care by attracting a beta male caregiver to the brood, which will be more likely to succeed when she selects wedge-shaped nest sites.

4. DISCUSSION

In several fish species without parental care, females may prefer group spawning, which induces multiple paternity and may ensure fertilizing success (reviewed in Taborsky 2008). However, in species where males provide brood care, females usually avoid multiple paternity to ensure the male provides brood care or to avoid filial-cannibalism by her mate (e.g. Lindström 2000). Our study is unique in showing that females may prefer to induce multiple paternity by spawning site selection and thereby gain paternal care of multiple males. Our results show that females of

J. transcriptus prefer wedge-shaped spawning sites only when both alpha and beta males are available, but not when either male is singly available. This strongly suggests that female nest choice in *J. transcriptus* is strategic and relates directly to attracting both potential caregivers to her nest. Furthermore, females in trios may carefully choose egg deposition sites, and thereby influence the paternity distribution of her two mates. In this way, both males were likely to sire eggs, but their actual siring success was not related to the level of direct brood care provided to the brood, similar to behaviour found in other cooperatively breeding cichlids (Heg *et al.* 2008) and birds (e.g. Hatchwell *et al.* 2002; Canestrari *et al.* 2005; Williams & Hale 2008). Cooperative systems, where parentage success was positively related to the level of paternal care, have also been reported (e.g. Scott & Williams 1993; Whittingham & Dunn 1998). Nevertheless, paternity might have affected other types of paternal care (e.g. Buchan *et al.* 2003), not tested in this study.

Cooperatively breeding cichlid females are not likely to acquire other benefits of multiple mating beyond those gained from direct or indirect paternal care. For instance: (i) females cannot use multiple mating to reduce infanticide (e.g. Heistermann *et al.* 2001; East *et al.* 2003; Wolff & Macdonald 2004), as this is mainly performed by dominant females towards the broods of subordinate females (Heg & Hamilton 2008); (ii) trios in our study were all unrelated, so females did not need to avoid inbreeding (e.g. Pusey & Wolf 1996; Koenig *et al.* 1998; Cooney & Bennett 2000; Cockburn *et al.* 2003; Griffin *et al.* 2003) and ensure fertilization by mating with multiple males (see review in Simmons 2005). Moreover, reproductively capable group members are generally unrelated in cooperatively breeding cichlids in nature, so females may not have been selected to avoid inbreeding (Awata *et al.* 2005; Dierkes *et al.* 2005; Stiver *et al.* 2005, 2008; M. Kohda, S. Awata, H. Munehara, T. Takeyama, T. Takahashi, K. Watanabe, M. Hori & D. Heg 2004, unpublished data), and (iii) females in trios also do not need to mate with the beta males to avoid harassment by these males (e.g. Sterck *et al.* 1997), because females were always larger than, and dominant over, these beta males (rather, females would potentially risk harassment by their alpha male if they mated with the beta male).

In principle, female control could also be used to bias paternity to the most vigorous caregivers, which in our study was usually the beta male, to obtain more paternal care (Houston *et al.* 1997; Birkhead 1998; Li & Brown 2002; Hamilton & Heg 2007; Rubenstein 2007). However, if a trio female biases paternity to her beta male, she will face a sexual conflict over paternity distribution with her alpha male (e.g. Arnqvist & Rowe 2005; Houston *et al.* 2005), and might risk retaliation by her alpha male (e.g. eviction). However, our results show that trio females of *J. transcriptus* could potentially spawn eggs at much deeper sites than they actually do (based on how deeply they can enter the wedges), so that beta males might completely fertilize the clutch at the expense of the alpha males. It is likely that it pays females to give some paternity to the lesser, alpha male caregivers, for example, to reduce alpha male harassment (Davies *et al.* 1996; Harada & Iwasa 1996), and to ensure alpha males provide other types of assistance through

predator defence and nest site guarding (Awata & Kohda 2004; Awata *et al.* 2005). As a balance of costs and benefits, or as a resolution of sexual conflict (Arnqvist & Rowe 2005), females in trios of *Julidochromis* appear not to bias the paternity to one male only, and on average reproductive skew was low (Johnston 2000; Cant & Reeve 2002). Our study is, to our knowledge, the first to document how nest site choice may mediate these effects.

Female control of paternity may also be mediated by cryptic female choice (Birkhead & Møller 1993; Eberhard 1996), sneaky copulations with the less competitive males (Stacey 1982; Burke *et al.* 1989; Davies *et al.* 1996) or any other mechanism that influences relative copulation frequencies of males and relative numbers of sperm reaching the eggs. Whereas females stand to gain from an additional beta male through increased paternal care, alpha males will incur a substantial cost through reduced paternity. However, this cost may be compensated for if beta males increase the total reproductive output of the group through offspring survival (Awata *et al.* 2005), if beta males release alpha males from duties allowing alpha males to mate with multiple females (polygyny, Awata & Kohda 2004; Awata *et al.* 2005, 2006), and if females increase their clutch size in the presence of a beta male (Reeve & Keller 2001; Hamilton & Heg 2007).

Our results also indicate that there should be a sexual conflict over group membership in *J. transcriptus*, which will be mediated by the body sizes and body size differences between the female and her male(s); and the availability of wedge-shaped nest sites. These factors together will determine the mating system of these cichlids as follows. If females are relatively small, larger males are likely to enter her territory and dominate. In such cases, females will mate monogamously, except when they have a wedge-shaped nest site available to attract a beta male into cooperative polyandry (like in our experiments). If a female is relatively large, she may physically dominate all males in the area. Depending on the number and sizes of males she can dominate or attract, she can either mate monogamously (like in our experiments with a single beta male) or mate with multiple smaller males each having its own nest, that is, so-called 'classical polyandry' (Yamagishi & Kohda 1996; Awata & Kohda 2004; Awata *et al.* 2005, 2006). The ability to attract or aggressively reject certain males from her nesting sites through a female body size advantage can be regarded as another type of female control (Eberhard 1996; Bertrams & Margalida 2004). If she does not have this advantage, we showed that females could still impose some control by nest site selection. We have shown that experimental studies of female control of group membership and paternity are needed to gain a deeper understanding of vertebrate cooperative breeding systems, as it may affect, for example, reproductive skew, intra- and inter-sexual conflicts, and the mating system.

All experiments were conducted in compliance with the guideline of the Animal Care and Use Committee of Osaka City University and the Japan Ethological Society.

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