

Sexual selection promotes colonial breeding in shell-brooding cichlid fish



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Colonial species breed in densely aggregated territories containing no resources other than nest sites. This behaviour is usually explained by natural selection, for instance through benefits resulting from reduced predation risk. An alternative hypothesis suggests that, as in lek breeding systems, sexual selection may be responsible for the aggregation of competitors, driven by an increased potential for female mate choice among closely assembled males. *Lamprologus callipterus*, a shell-brooding cichlid fish of Lake Tanganyika, provides an ideal test case for the utility of lek evolution models to explain colonial breeding, because breeding territories are established by males before pairing. Large males collect and defend empty snail shells that are then chosen by females for breeding. We checked for a potential influence of sexual selection on colonial breeding in *L. callipterus* by testing predictions of the hot-shot and female preference hypotheses of lek mating models. In the field, we found that territories of larger males were more centrally located and that females preferred to breed with males surrounded by many neighbours, two findings that are consistent with lek mating models. Female preference suggests that sexual selection affects colonial breeding in *L. callipterus*, which implies an influence of sexual selection on the evolution of colonial breeding at large.

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There is ongoing debate about why individuals breed in colonies (Danchin & Wagner, 1997; Höglund & Alatalo, 1995; Neff & Pitcher, 2008; Sachs, Hughes, Nuechterlein, & Buitron, 2007). In breeding colonies, individuals aggregate and defend nest sites or small territories that contain no other resources (Danchin & Wagner, 1997; Perrins & Birkhead, 1983; Siegel-Causey & Kharitonov, 1990; Wittenberger & Hunt, 1985). This breeding pattern is widespread in vertebrates ranging from fish (Gross & MacMillan, 1981; Tyler, 1995) to reptiles (Trillmich & Trillmich, 1984) and marine mammals (Terhune & Brillant, 1996). Most prominently, this breeding pattern is prevalent in seabirds, in which 95% of all species breed in colonies (Brown, Stutchbury, & Walsh, 1990; Wittenberger & Hunt, 1985). Several evolutionary mechanisms have been proposed to explain colonial breeding. 'Conspecific models' assume that the fitness benefits of colonial breeding are gained from nesting near conspecifics (Sachs et al., 2007): nesting sites should be aggregated

if colonial breeding, for instance, increases foraging efficiency (Beauchamp, 1999), facilitates information transfer (Barta & Giraldeau, 2001; Buckley, 1997), reduces the predation risk for offspring (Gross & MacMillan, 1981; Picman, Pribil, & Andre, 2002; Post, 1998; Schaedelin, Fischer, & Wagner, 2012; Tyler, 1995) or enables parasitic reproduction (Picciulin, Verginella, Spoto, & Ferrero, 2004). 'Habitat models', on the other hand, assume that the fitness benefits of colonial breeding are gained through the characteristics or location of the colony site (Sachs et al., 2007), mainly when suitable breeding habitats are limited (Johnson & Walters, 2011; Kiester & Slatkin, 1974; Wittenberger & Hunt, 1985). Either way, these benefits must outweigh the potential costs of colonial breeding, such as increased parasite transmission, potential infanticide and competition for mates (Danchin & Wagner, 1997; Kiester & Slatkin, 1974).

In lek mating systems, in contrast, males aggregate at display arenas which females visit solely for the purpose of mating (Höglund & Alatalo, 1995; Kiester & Slatkin, 1974; Loiselle et al., 2007). Sexual selection is usually held responsible for the evolution of leks. First, the 'hot-shot model' assumes that certain males are preferred by females over other males, and that subordinate or less attractive males aggregate around such 'hot-shots' in order to increase their mating success (Bednekoff, 2002; Beehler & Foster,

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1988; Westcott & Smith, 1997). Second, the 'female preference model' assumes that females benefit from clumped male territories either because they can better estimate relative mate quality (Wagner, 1998) or because males of high quality tend to be found in larger aggregations (Höglund & Alatalo, 1995). According to this model, females preferentially settle in large aggregations (Fletcher & Miller, 2006). Third, the 'hot-spot model' (Bradbury, Gibson, & Tsai, 1986) hypothesizes that breeding aggregations are a result of males settling in regions with high female abundance (Höglund & Alatalo, 1995; Westcott, 1997). Females may have large overlapping home ranges relative to male territory size or well-defined travel routes (Bradbury et al., 1986; Westcott, 1997). Males aggregate to increase pairing success, and extrapair copulations (EPCs) may occur as a by-product of the aggregation (Fletcher & Miller, 2006). Finally, the 'kin selection model' assumes that subordinate males gain inclusive fitness benefits by aggregating among related males (Bradbury & Gibson, 1983; Kokko & Lindström, 1996). Only males that are related to the dominant male aggregate (Fletcher & Miller, 2006).

The hidden lek hypothesis of colonial breeding provides an alternative explanation, assuming that intersexual selection (female choice) may be responsible for the aggregation of competitors also in nonlekking species that engage in paternal care (Wagner, 1998). This hypothesis is an extension of the 'EPC hypothesis' (Wagner, 1993), which originated from the discovery that socially monogamous birds of both sexes may pursue EPCs (Birkhead & Møller, 1992). The hidden lek hypothesis assumes similar mechanisms to those in lekking species to be responsible for an aggregation of nests in a colony (Bradbury, 1981), such as the 'hot-shot', 'female preference' 'hot-spot' and 'kin selection' models (Fletcher & Miller, 2006). Five criteria are required for breeding colonies to classify as hidden leks (Fletcher & Miller, 2006), four of which focus on the role of extrapair matings for the accumulation of territories: (1) The species must be socially monogamous or polygynous, where females develop pair bonds with males that provide some form of paternal care; (2) territorial males aggregate, forming arenas where EPCs occur; (3) females seek EPCs and are able to select extrapair mates; extrapair males (4) provide no parental care and (5) do not monopolize resources required by females. A study of colony formation in the socially monogamous least flycatcher, *Empidonax minimus*, revealed that some predictions of the hidden lek hypothesis indeed apply in that species (Tarof, Ratcliffe, Kasumovic, & Boag, 2005).

The current consensus is that colonial and lek mating systems differ distinctively in the parental care system: lekking males provide no parental care while colonial males typically provide substantial and usually essential care. The role of sexual selection is assumed to be restricted to the evolution of leks and its importance is neglected as a driver of colonial breeding, because in many colonial species, breeding pairs choose a nest site together, i.e. after mate choice and pairing. However, if aggregated territories are founded by males only, there is high potential for sexual selection to operate. For example, in bluegill sunfish, *Lepomis macrochirus* (Gross & MacMillan, 1981), the cichlid *Lamprologus callipterus* (Sato, 1994), boat-tailed grackles, *Quiscalus major* (Post, 1998), Mediterranean damselfish, *Chromis chromis* (Picciulin et al., 2004), lesser kestrel, *Falco naumanni* (Calabuig, Ortego & Aparicio 2011) and western sandpipers, *Calidris mauri* (Johnson & Walters, 2011), males establish a territory first and females settle later. In all these cases, sexual selection mechanisms that are responsible for lek evolution might operate as well, but they have not been explicitly addressed. In boat-tailed grackles, for instance, the difference in reproductive success between colonial and solitary females is explained by the males' defence of colonies from predatory birds (Post, 1998). Breeding colonies of lesser kestrels apparently result

from neutral colony selection by first-year birds based on nest site availability (Calabuig et al., 2011). Bluegill sunfish colonies were assumed to result from reduced brood predation through nest aggregations (Gross & MacMillan, 1981), whereas in the Mediterranean damselfish, colonial nesting was shown to facilitate parasitic reproduction (Picciulin et al., 2004).

Lamprologus callipterus, a cichlid fish endemic to Lake Tanganyika, provides an ideal opportunity to explore predictions derived from lek evolution models to explain colonial breeding. Here, unlike in most colony breeders, males establish their aggregated territories first, i.e. before pairing. Therefore, a direct influence of females on colony formation at the time of territory establishment is very unlikely. Instead, males may be selected to aggregate prior to female arrivals in order to subsequently increase their mating success. Females choose males, established nests or shells contained therein for mating, which yields a high potential for female choice to operate after initial colony formation. The breeding system of *L. callipterus* seems to conform to a number of predictions from models of lek mating systems. Males defend territories in clusters (hereafter referred to as 'nest'), which contain no resources other than empty snail shells attracting females for mating (Mitchell, Wirtz Ocana, & Taborsky, 2014; Nakai, Yanagisawa, Sato, Niimura & Gashagaza 1990). However, in contrast to a pure lek, females stay in the male territory after mating, and males indirectly care for the brood by antipredator defence (Maan & Taborsky, 2008; Sato, 1994). Hence, the mating system of *L. callipterus* conforms also to resource defence polygyny (Emlen & Oring, 1977). Territories of bourgeois males consist of accumulated gastropod shells of the species *Neothauma tanganicense* or *Paramelania damoni*, which serve as exclusive breeding substrate for females (Gashagaza, Nakaya, & Sato, 1995; Sato, 1994; Sato & Gashagaza, 1997; Schütz & Taborsky, 2000). Shells are collected nearby, or stolen from neighbouring nests (Maan & Taborsky, 2008; Mitchell et al., 2014; Sato, 1994). Females perform brood care for 10 to 14 days within these shells (Sato, 1994).

Throughout the breeding area, sneaker males of all sizes try to steal fertilizations from territory owners (Sato, 1994; Schütz & Taborsky, 2000; Taborsky, 1998, 2001). Additionally, small dwarf males attempt to gain fertilizations by entering shells with a spawning female inside (Sato, Hirose, Taborsky, & Kimura, 2004; Taborsky, 1998, 2001) and releasing sperm from within the shells (Wirtz Ocana, Meidl, Bonfils, & Taborsky, 2014). Sneakers and dwarf males do not show any parental care (Taborsky, 2001), and most sneaker males are too small to claim breeding territories, because they cannot manage to carry shells (Schütz & Taborsky, 2005). In contrast to females breeding on hidden leks, which are expected to prefer aggregated males to improve the potential for EPCs (Tarof et al., 2005; Wagner, 1993), in *L. callipterus* females are not able to actively seek extrapair matings, since egg deposition and fertilization takes place inside the snail shell (Schütz, Heg-Bachar, Taborsky, & Heg, 2012; Taborsky, 2001). The only scope for female choice regarding parasitic males would occur after a female has already chosen a nest and entered a shell, i.e. during her spawning. A dwarf male attempting to enter a shell for spawning needs to pass the female, which can resist this attempt as this passing usually requires a slight backward movement of the female towards the shell entrance (Sato et al., 2004; D. Schütz, S. W. Ocana, M. E. Maan & M. Taborsky, personal observations).

In this study, we examined the influence of sexual selection on colonial breeding in *L. callipterus* by testing some predictions of lek mating models. In this species nesting males aggregate after a dispersal period extending well over a year (Schütz, Parker, Taborsky, & Sato, 2006), during which individuals mix and roam about in schools (Sato et al., 2004; Taborsky et al., n.d.), just as females do between successive broods (Schütz et al., 2006).

Therefore relatedness is highly unlikely to affect settlement patterns, and the kin selection model can be excluded. Since feeding schools of nonbreeding fish have no well-defined home ranges or travel routes (D. Schütz, S. W. Ocana, M. E. Maan & M. Taborsky, personal observation), the hot-spot model is also unlikely to apply. Therefore, we focus on predictions of the hot-shot and female preference models. Earlier field studies showed that females did not choose particular males when nest density was high (Schütz & Taborsky, 2005), but seemed to prefer to mate with larger males when nest density was low (Maan & Taborsky, 2008). It is not clear, however, whether females directly prefer large males or males with many neighbours. Hence, we tested in the field whether male size relates to their nest location in a colony, i.e. whether large males have more neighbours (hot-shot model). Furthermore, we tested whether females preferentially settle in large aggregations of nests, i.e. whether they prefer males with many neighbours (female preference model).

METHODS

Study Population

We studied a population of *L. callipterus* that was located at Kasakalawe Point near Mpulungu, Zambia (08°45'S, 31°04'E). Breeding colonies of *L. callipterus* are temporary, with large differences in population density, colony size and nest density within colonies between years. To take this variance into account, we analysed two data sets, one from 1997, when population density was low, and one from 2005, when population density was high. In both years, all territories of *L. callipterus* at Kasakalawe Point were mapped. All observations were done by SCUBA diving. For catching of fish a fence net or hand nets were used.

In 1997, we studied *L. callipterus* from 15 January to 4 March and found only one breeding colony with 28 nests. The nests were located between 8 and 12 m depth. This colony contained all *L. callipterus* nests found along a stretch of 500 m of coastline between 4 and 15 m water depth, i.e. within an area of approximately 125 000 m². From 16 September to 27 November 2005, we studied *L. callipterus* at the same place and found two colonies (named 'West' and 'East') within the same area. They contained 80 and 53 nests, respectively, and were approximately 200 m apart. The nests were located between 9 and 15 m depth and were the only breeding sites of *L. callipterus* in the above-mentioned area during that period.

Data Collection

In *L. callipterus*, as nest density is highest in the centre of a colony, males with more neighbours occupy more central territories. To quantify the density of the area around a nest, all neighbouring nests within a radius of 3 m around each nest were counted. The distance to the closest nest was measured to the

nearest 10 cm for all nests that persisted for the whole observation period ($N = 28$ in 1997 and $N = 118$ in 2005). The distance to the closest nest correlated highly with the number of nests within a radius of 3 m in both years (Spearman correlations: 1997: $r_s = -0.543$, $N = 28$, $P = 0.003$; 2005: $r_s = -0.611$, $N = 118$, $P < 0.001$); therefore we report only one of these measures (nests within 3 m) as an estimate of nest density.

Catching and measuring nest males can involve considerable disturbance, which might influence results if males subsequently leave or females are deterred. Therefore, we haphazardly chose a subset of males to determine their sizes to the nearest mm underwater with a measuring plate (standard and total length, SL and TL, $N = 17$ in 1997 and $N = 15$ in 2005).

In 1997, we counted breeding females for each nest every day. Owing to the large nest number in 2005, in that year we randomly chose a subset of all nests every day to estimate the number of breeding, spawning and visiting females (mean \pm SD: 14.6 ± 8.3 nests checked per day, with an average interval of 9.8 ± 7.1 days between subsequent observations of individual nests). We aimed to check every nest once during each moon phase (see below). After counting breeding and spawning females, we observed each focal nest for 4 min, and all females within a nest, which were not spawning or breeding but inspecting shells, moving in the nest, sitting on shells or on the ground within nests, were categorized as visiting females. To avoid counting individual females more than once during the observation period, each visiting female was individually tracked after her first visit and not recounted when she revisited the nest.

For *L. callipterus*, a lunar synchronization of breeding activity has been reported (Nakai, Yanagisawa, Sato, Niimura, & Gashagaza, 1990; Maan & Taborsky, 2008). To account for this periodicity, we divided the lunar month into four periods: 'full moon', 'waning moon', 'new moon' and 'waxing moon' (7 days per period). Most spawning, breeding and visiting females in nests were found around full moon (Table 1).

Statistics

The distributions of all data were tested for normality with Kolmogorov – Smirnov normality tests. If data differed from a normal distribution ($P < 0.1$), nonparametric statistics were used. As a measure of male reproductive success, we counted breeding females per nest at full moon. In 1997 as each nest was checked every day, we used the number of breeding females on the day of full moon (23 January 1997, $N = 20$). In 2005 as each nest was only checked once during each moon phase, we used the number of breeding females during the 7-day period around full moon (13–19 November 2005, full moon on 16 November 2005, $N = 50$). As a measure of male reproductive skew, we determined the frequency distribution of the numbers of breeding females per male at full moon in both years. For both years, we used Spearman rank correlations to test whether (1) male size and (2) the number of

Table 1
Female abundance during different moon phases

	Spawning females			Breeding females			Visiting females		
	Median	Quartiles	<i>N</i>	Median	Quartiles	<i>N</i>	Median	Quartiles	<i>N</i>
Full moon	1.0	0.0–2.0	88	6.0	3.25–9.75	80	0.0	0.0–1.25	34
Waning moon	0.0	0.0–1.0	102	3.0	1.0–7.0	101	0.0	0.0–0.0	43
New moon	0.0	0.0–1.0	62	2.5	1.0–5.0	68	0.0	0.0–1.0	38
Waxing moon	1.0	0.0–2.0	50	1.0	0.0–3.0	45	0.0	0.0–0.0	28
KW statistic	$\chi^2=37.694$, $P<0.001$			$\chi^2=52.206$, $P<0.001$			$\chi^2=7.609$, $P=0.055$		

Numbers (medians and quartiles) of spawning, breeding and visiting females per nest during the four moon phases in all nests (2005, 'high-density nests' and 'low-density nests' combined). Results of Kruskal–Wallis tests (KW) comparing the female numbers between moon phases are given at the bottom; all $df = 3$.

breeding females at full moon correlated with the number of nests within a radius of 3 m. In 2005, for each of the four moon periods, Spearman rank correlations were used to relate the number of neighbours within a radius of 3 m to the number of spawning, breeding and visiting females per nest. In addition, we differentiated between ‘high-density nests’ (nearest neighbour ≤ 2 m) and ‘low-density nests’ (nearest neighbour > 2 m; for details see the Appendix).

Combining data from both years, we tested whether female preference for high-density nests can be attributed to female preference of larger males assembling at higher densities. We standardized our data ($(x - \text{mean})/\text{SD}$) to bring the variables into proportion with each other and to improve model interpretation. We used a linear model (LM) with the number of breeding females as the dependent variable, and population density (year), nest density (the number of neighbours within a 3 m radius), male size (TL), and the interaction between male size and nest density as covariates. This model gives estimates for the effects of male size and nest density when the other variable is at average and allows us to compare the influences of both variables on the number of breeding females. As collinearity may affect the outcome of the presented model, we additionally checked for the influence of male size and nest density on the number of breeding females in two separate linear models using the original data. For these and the other LMs used in this study the residuals were checked for potential deviations from normality, and the assumptions underlying this type of analysis were not violated.

To test for female mate copying, in the year with high population density we tested whether the numbers of (1) visiting and (2) spawning females were influenced by nest density and the numbers of females already breeding in a nest. We used univariate linear models with the number of (1) visiting and (2) spawning females as dependent variables, and the number of neighbours within a 3 m radius and the number of breeding females as covariates. Additionally, we used Spearman rank correlations to check whether the presence of breeding females correlated with the number of females visiting a nest separately for all four moon phases. For all analyses, two-tailed tests were applied.

Ethical Note

The field work at Lake Tanganyika was conducted with the permission of the Fisheries Department of the Ministry of Agriculture and Co-operatives of Zambia. When fish were caught and measured under water, we immediately released them at the same place where we had caught them. No fish was harmed by our handling.

RESULTS

How Big is Male Mating Skew?

In both years, male mating success was moderately skewed, with few males receiving no breeding females in their nests (15% in 1997 and 6% in 2005; Fig. 1).

Do Larger Males have more Neighbours?

In the year with low population density (1997), the distance to the nearest neighbouring nest was at least 2 m (range 2–15 m), and males had a maximum of two neighbours within a radius of 3 m (median = 1; range 0–2; Fig. 2). In the year with high population density (2005), the distance to the nearest neighbouring nest varied between 0.5 and 22 m ($N = 108$ nests) within colonies, and males had up to 13 neighbours within a radius of 3 m (median = 4;

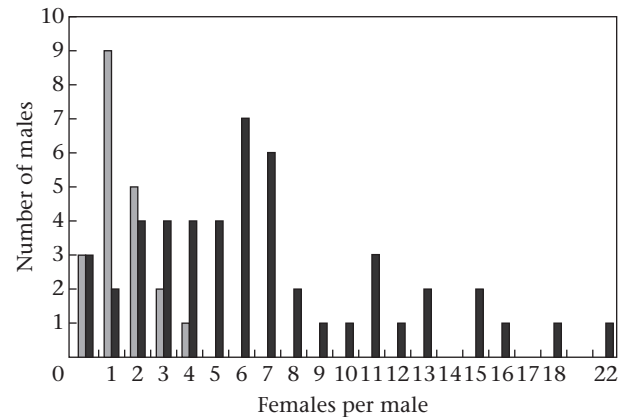


Figure 1. Frequency distribution of the numbers of breeding females per male at full moon in years with low population density (grey bars) and high population density (black bars).

range 0–13; Fig. 2). In both years, larger males had more neighbours within a radius of 3 m than smaller males (Spearman correlations: 1997: $r_s = 0.62$, $N = 17$, $P = 0.008$; Fig. 3a; 2005: $r_s = 0.832$, $N = 15$, $P < 0.001$; Fig. 3b).

Do Females Choose Nests with more Neighbours?

In the year with low population density, there was no significant correlation between the number of breeding females and the number of nests within a radius of 3 m at full moon (Spearman correlation: $r_s = -0.15$, $N = 19$, $P = 0.539$). In contrast, in the year with high population density, there was a positive correlation between the number of neighbours within a radius of 3 m around the nest and the number of breeding females (Spearman correlation: $r_s = 0.304$, $N = 80$, $P = 0.006$) and visiting females (Spearman correlation: $r_s = 0.447$, $N = 34$, $P = 0.008$) in a nest during the full moon period. At new moon, also more breeding females were found in nests with more neighbours (Spearman correlation: $r_s = 0.266$, $N = 68$, $P = 0.029$), whereas at other moon phases these correlations were nonsignificant ($0.114 < P < 0.98$). For a comparison between ‘high-density nests’ and ‘low-density nests’ in that year see Appendix Tables A1 and A2.

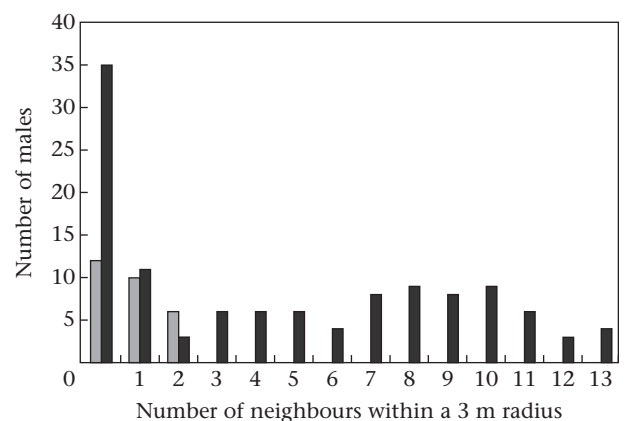


Figure 2. Frequency distribution of the numbers of neighbours within a radius of 3 m in years with low population density (grey bars) and high population density (black bars).

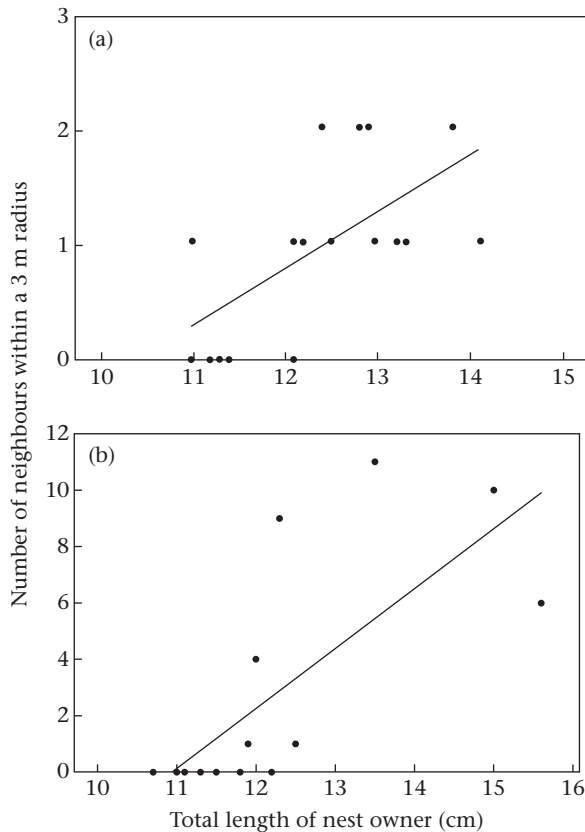


Figure 3. Relationship between the total length of nest males and the number of neighbours, and regression lines: (a) low population density (1997), $N = 17$ and (b) high population density (2005), $N = 15$. Note the different scales on the y-axis.

Do Male Size and Nest Density Influence Female Numbers?

In both years combined, the number of breeding females per nest was significantly influenced by population density (year), nest density (the number of nests within a 3 m radius) and male size (TL; LM: $r^2 = 0.59$, $F_4 = 9.547$, $P < 0.001$). Population density, nest density and the interaction between male size and nest density significantly determined the number of breeding females, but male size did not (see Table 2). This means that for an average-sized male, nest density had a strong effect, whereas for a male with an average nest density, body size had only a very weak effect on the number of breeding females. The estimate of the interaction between nest density and male size was negative, which shows that with increasing numbers of neighbours, body size becomes less important, and with increasing male size, the number of neighbours becomes less important. When checking for the influence of male size and nest density on the number of breeding females separately, we found that only nest density showed a significant effect (Table 3).

Table 2
Effects on the number of breeding females: combined model

Coefficient	Estimate	SE	<i>t</i>	<i>P</i>
Year	1.05	0.28	3.73	<0.001
Nest density	1.09	0.27	4.09	<0.001
Male size	0.007	0.17	0.04	0.97
Nest density * male size	-0.60	0.16	-3.854	<0.001

Results of linear model estimating effects of population density, nest density, male size, and the interaction of male size and nest density on the number of breeding females.

Table 3
Effects on the number of breeding females: separate models

Coefficient	r^2	<i>df</i>	<i>F</i>	<i>P</i>
(1) Total model	0.296	2	5.876	0.007
Year		1	9.43	0.005
Male size		1	2.669	0.114
(2) Total model	0.364	2	7.997	0.002
Year		1	5.640	0.025
Nest density		1	5.941	0.021

Results of two linear models estimating the effects of (1) population density (year) and male size and (2) population density (year) and nest density on the number of breeding females.

Do Females Copy the Mate Choice of Others?

In the year with high population density, our analysis (LM: $r^2 = 0.189$, $F_2 = 16.178$, $P < 0.001$) suggested that the number of females visiting a nest was influenced significantly by the number of females already breeding there ($F_1 = 30.596$, $P < 0.001$), but not by nest density ($F_1 = 0.428$, $P = 0.514$). In contrast, the number of spawning females (LM: $r^2 = 0.031$, $F_2 = 4.278$, $P = 0.015$) was significantly influenced by nest density ($F_1 = 8.28$, $P = 0.004$), but not by the number of females already breeding in the nest ($F_1 = 0.987$, $P = 0.321$). When separated for moon phases, the presence of breeding females was positively correlated with females visiting a nest around full moon (Spearman correlation: $r_s = 0.429$, $N = 34$, $P = 0.011$) and in the waning moon period (Spearman correlation: $r_s = 0.333$, $N = 43$, $P = 0.029$), but not in the waxing moon period (Spearman correlation: $r_s = 0.342$, $N = 28$, $P = 0.075$) and around new moon (Spearman correlation: $r_s = -0.0199$, $N = 37$, $P = 0.911$).

DISCUSSION

Two models of lek breeding were supported by our results: the hot-shot and the female preference models. Large males occupied central positions in the aggregation of nests, and females preferred to breed in nests at high densities. This suggests a significant influence of sexual selection on the evolution of colonial breeding in *L. callipterus*. We agree with Jiguet and Bretagnolle (2006) that these two models are somewhat complementary, promoting male clustering and the maintenance of leks. We argue that these mechanisms may also promote colonial breeding, especially in species in which males settle before pairing (see examples given above). Some evidence that sexual selection mechanisms operating in lek breeding may also apply to colony breeders exists also in the banded damselfish, *Abudefduf abdominalis* (Tyler, 1995) and in the blue-black grassquit, *Volatinia jacarina* (Almeida & Macedo, 2001).

In leks, male mating success is usually strongly skewed, with one or only a few males obtaining all fertilizations (Ryder, Parker, Blake, & Loiselle, 2009). For example, in the lekking lance-tailed manakin, *Chiroxiphia lanceolata*, nonalpha males can hardly reproduce (DuVal & Kempnaers, 2008) and in black grouse, *Tetrao tetrix*, most males fail to successfully copulate during their lifetime (Kervinen, Alatalo, Lebigre, Siitari, & Soulsbury, 2012). In contrast, in *L. callipterus* most nest males were able to attract at least one female; hence male reproductive skew appears to be smaller than in typical lek mating species.

In accordance with predictions of the hot-shot model, in *L. callipterus* large males were located in the centre of male aggregations. Large nest owners, which were shown to be superior competitors (Maan & Taborsky, 2008; Schütz & Taborsky, 2005), had more nests in their vicinity than smaller males in both years of study and at greatly different population densities. This may

suggest that the largest, most competitive males settle first and smaller, less competitive males follow. Large males might also better defend their nest against predators, which could be another reason why smaller males settle near them. In least flycatchers, which form dense territorial clusters, central males arrived earlier, were heavier and in better body condition than peripheral males (Tarof et al., 2005).

In accordance with the female preference model of lek evolution, females preferentially settled in denser aggregations. In the year with high population density, there was a positive correlation between the number of neighbours and the number of breeding and visiting females per nest. However, in the year with low population density, nest density did not correlate with the number of breeding females, which is probably due to the relatively large distances between neighbouring nests (≥ 2 m) and the low numbers of neighbours in this year. When searching for suitable breeding substrates and comparing nests, females cover large distances. If nests are further apart, this entails high travel costs including energy expenditure and predation risk, and comparing males becomes more difficult. Thus, the advantages of reduced travel costs from searching in densely aggregated nests are probably lacking when population density is low. We therefore assume that in *L. callipterus*, female choice is strongly influenced by both population density and local nest density.

In the year with high population density, more females were breeding at high local nest densities, even though male body size and the number of suitable shells per nest did not differ between the two local densities (see Appendix). This pattern confirms the preference of females for areas with densely aggregated males. The numbers of spawning and visiting females did not differ between the two local nest densities, which could be due to the lower chance to detect these females. 'Visiting' and 'spawning' are more transient activities than 'breeding', which involves brood care lasting for 10–14 days (Schütz & Taborsky, 2005). As visiting females enter the nest for only short periods of time, they could sometimes be missed during the 4 min observation periods. Spawning is often aborted, especially during the first hour of male spawning, when the female is not yet laying eggs (Schütz et al., 2012). Thus, the count of spawning females is not a very good estimate of male success. Instead, the count of breeding females is the most reliable measure of female preference.

A combined analysis of female settlement patterns including data from both years suggested that mainly nest density, and not male size, influenced female preference. A linear model with the standardized values showed a strong and highly significant effect of the number of neighbours: average-sized males would gain 1.09 breeding females if they had one more neighbour. In contrast, the effect of male size on the number of breeding females was insignificant and in fact almost zero: with an increase of 1 cm in total length, males with an average number of neighbours would only receive 0.007 more females. The effect of the interaction between nest density and male size was negative and highly significant, which shows that an increase in one of the two variables leads to a decreasing effect of the other variable on the number of breeding females. The results of the models dealing with each variable separately support the reported effects. Thus, the advantage for females choosing nests with many neighbours is not triggered by the fact that large males are occupying nests in dense areas. Previous experiments showed that females did not prefer large males to small ones when given the choice, but rather use features of their shell collections to decide where to breed (Mitchell et al., 2014; Schütz & Taborsky, 2005). We therefore conclude that females mainly choose a spawning territory with males that have many neighbours, and that male size is of secondary importance. However, the comparison between study years suggests that population

density needs to be high enough for nest density effects to emerge. Ultimately, one could argue that female preference influences nest site choice of *L. callipterus* males during colony formation, even if females select males only after the latter have settled, i.e. this effect of female choice on male settlement patterns occurs a posteriori.

The behaviour of visiting females might indicate a potential effect of mate choice copying. In the year with high population density, the presence of already breeding females positively correlated with the number of females visiting a nest during the most active spawning period, i.e. around full moon. However, the number of spawning females was significantly influenced by nest density and not by the number of breeding females. Thus females seem to prefer to visit nests containing many breeding females, but then decide to spawn and breed in nests with many neighbours. Since more females are breeding in nests with many suitable shells than in nests with few suitable shells (Schütz & Taborsky, 2005), the correlation between visiting and breeding females might be due to more suitable shells in nests containing more breeding females. Previous experiments refuted the hypothesis that females copy the nest choice of other females (Schütz & Taborsky, 2000). In ruffs, *Philomachus pugnax* (Lank & Smith, 1992) and black grouse (Alatalo, Höglund, Lundberg, & Sutherland, 1992), larger leks had more female visits and copulations, leading to a higher average male mating success. In *L. callipterus*, we cannot test for an influence of colony size on female preference and male mating success due to limited data, but we assume that colony size might positively influence these parameters as well.

Female choice of nests with many neighbours may yield several benefits; for instance it may lower their travel costs when searching for mates, and it may facilitate their evaluation of male, nest and shell quality. Before spawning, females move from nest to nest to inspect potential shells, nests and males (Mitchell et al., 2014; D. Schütz, S. Wirtz, Ocana, M. E. Maan & M. Taborsky, personal observations). More females were visiting high-density than low-density nests in the period of highest spawning activity (around full moon; see Appendix), which suggests that comparing suitable shells and males may be easier in high-density than in low-density nests. Another potential advantage of breeding in dense colonies is the reduction of predation risk for females and their offspring (nest defence hypothesis, Gross & MacMillan, 1981; Picman et al., 2002; Schaedelin et al., 2012). Since in *L. callipterus* the female and her brood are located deep inside the shell, offspring predation is not very frequent (D. Schütz, S. W. Ocana, M. E. Maan & M. Taborsky, personal observations). However, reduced travel distances between territories in a dense colony may reduce the predation risk for females during mate search (see Alem & Greenfield, 2010).

Breeding in dense colonies may also entail potential costs to females. In *L. callipterus*, settling in areas with many nests close by can increase the risk that the shell in which a female raises her offspring is stolen by a neighbouring male, which is typically followed by expulsion from the shell and offspring loss (Maan & Taborsky, 2008). Most naturally occurring shell stealing is by bourgeois nearest neighbours (Maan & Taborsky, 2008). By choosing a nest with a large owner, this risk might be reduced as large males are in general more aggressive and able to hold a territory longer (Maan & Taborsky, 2008; Schütz & Taborsky, 2005). Costs of breeding in dense colonies have also been suggested in other species such as the common guillemot, *Uria aalge*, in which aggression towards unattended chicks often results in infanticide, particularly at high nest densities (Ashbrook, Wanless, Harris, & Hamer, 2010).

The mating and breeding pattern of *L. callipterus* suggests that colonial breeding can be subject to sexual selection. This has also been proposed by the hidden lek hypothesis, which assumes that colony formation in birds may result from female preference for

EPCs (Wagner, 1993, 1998). For breeding colonies to be classified as hidden leks it is required that females are able to actively seek EPCs (Fletcher & Miller, 2006). The mating pattern of *L. callipterus* excludes the possibility that females search for extrapair fertilizations. A female ready to spawn enters the shell completely, and the bourgeois and sneaker males release sperm into the shell opening, without direct contact with the female (Schütz et al., 2012). Females spawning in regions of high nest density might increase their chances of obtaining extrapair fertilizations from sneaker males, if sneakers preferentially search for spawning opportunities in regions with high nest density. However, a field study revealed that the number of spawning females per nest and day does not correlate with the number of sneakers and dwarfs entering a nest per day (Schütz, Tschirren, Pachler, Grubbauer & Taborsky, n.d.). Sneaker males are found mainly in feeding schools (Schütz et al., 2006) and the mean number of sneakers entering a nest per day is low (0.66; Schütz et al., n.d.). Sneakers seem to take opportunities to release sperm whenever possible, without comparing nests. Additionally, sneaker fertilizations are generally very rare in nature (<1%, Wirtz Ocana et al., 2014); thus selecting extra mates is an unlikely explanation for female preference of dense colonies in *L. callipterus*. On the contrary, females seem to avoid the participation of parasitic males if they can (Sato et al., 2004; D. Schütz, S. W. Ocana, M. E. Maan & M. Taborsky, personal observations), which, if anything, should rather reduce preference for dense nest areas. Thus, in *L. callipterus* female preference to spawn in dense colonies cannot be explained by a preference for extrapair fertilizations.

We therefore argue that mechanisms of sexual selection responsible for the formation of leks beyond the potential enhancement of extrapair mating can influence colonial breeding in *L. callipterus*. Such mechanisms may operate in colonial species more generally, i.e. also beyond increasing EPCs. This is especially apparent if territories and nests are founded by the sexually selected sex only, but it may also be important, yet more hidden, in other cases. We propose, therefore, to expand the hidden lek hypothesis beyond the function of facilitating extrapair matings.

Conclusions

Our results are consistent with both the ‘hot-shot’ and ‘female preference’ hypotheses of lek evolution. In the colonial breeder *L. callipterus*, the formation of aggregated nesting can be at least partly explained by sexual selection. Larger males had more neighbours than smaller ones, which means that they were in the centre of colonies. This is predicted by the hot-shot model. Particularly in the year with high population density, females preferred to mate and breed with males in denser aggregation areas, which is predicted by the female preference model. Regarding the importance of sexual selection for the generation of breeding aggregations, the two allegedly distinct reproductive systems characterized by the absence (lek mating) or presence (colonial breeding) of paternal care may in fact reflect different end points on a continuum. We suggest that models explaining lek evolution via sexual selection can also be used to test predictions of mating patterns in colony breeders, especially (but not only) when males settle first and females follow.

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APPENDIX

Comparison Between High- and Low-density Nests in 2005

Methods

In the year with high population density, we tested whether female choice is influenced by local nest density. Each nest was defined as either 'high-density nest' (nearest-neighbour distance <2 m; $N = 72$, with 36 each in the West and East Colonies) or 'low-density nest' (nearest-neighbour distance ≥ 2 m; $N = 61$, with 44 in the West and 17 in the East Colony, respectively). To obtain a measure of nest quality, we determined the number of shells suitable for breeding (intact shells >4.5 cm; Schütz & Taborsky, 2000) by measuring them along the axis from the apex to the outer part of the lip (Stresemann, 1992). Additionally, the total number of shells (i.e. including broken shells) was evaluated for a subsample of nests. Ten to 12 days after spawning, i.e. shortly

Table A1

Comparison of territories between high- and low-density nests

	High-density nests	Low-density nests	Test	<i>P</i>
Size of nest owners (cm)	10.17±1.26	10.0±1.18	$t_{40}=0.451$	0.655
Size of breeding females (cm)	4.96±0.34	5.0±0.27	$t_{28}=0.339$	0.736
Number of fry per brood	122.92±38.2	126.8±33.8	$t_{29}=0.290$	0.774
Suitable shells per nest	23.0 (16.5–30.0)	22 (16.25–28.75)	$U_{37,24}=423.0$	0.756
Spawning females per nest	0.0 (0.0–1.0)	1.0 (0.0–1.75)	$U_{170,132}=12313.5$	0.113
Breeding females per nest	4.0 (2.0–7.0)	2.0 (1.0–5.0)	$U_{169,125}=8105.0$	0.001
Visiting females per nest	0.0 (0.0–1.0)	0.0 (0.0–1.0)	$U_{69,74}=2448.5$	0.60

For normally distributed data (standard lengths of fish and number of fry produced) means ± SDs are shown. For non-normally distributed data, medians and interquartile ranges are shown. Tests were *t* tests (*t*) and Mann–Whitney *U* tests (*U*). The only significant difference is highlighted in bold.

Table A2

Comparison of female abundance between high- and low-density nests during the four moon phases

	Breeding females	Visiting females
Full moon	$U=566, N_1=44, N_2=35, P=0.028$	$U=77, N_1=14, N_2=20, P=0.027$
Waning moon	$U=896, N_1=61, N_2=40, P=0.024$	$U=227.5, N_1=20, N_2=23, P=0.934$
New moon	$U=372, N_1=44, N_2=24, P=0.043$	$U=214.5, N_1=21, N_2=17, P=0.294$
Waxing moon	$U=249.5, N_1=20, N_2=25, P=0.99$	$U=98, N_1=14, N_2=14, P=1.0$

Results of Mann–Whitney *U* tests to compare the numbers of breeding and visiting females between high- and low-density nests at different moon phases. Significant correlations are highlighted in bold (see Fig. A1).

before young left the shell, a subset of breeding females ($N = 30$) was transported to the surface and the female and her fry were carefully shaken out of the shells. The female was measured to the nearest mm and the young were counted. After measuring and counting, the fish were brought back to where we caught them. No fish were harmed by our handling.

We compared the body size of nest owners, the sizes of breeding females and the number of fry produced between low-density and high-density nests using Student's t tests, and the numbers of suitable shells, spawning, breeding and visiting females per nest using Mann–Whitney U tests. To test for a potential influence of the lunar period on the numbers of breeding and visiting females per nest found at high and low local nest densities, we used Mann–Whitney U tests.

Results

In the year with high population density, body sizes of nest owners, sizes of breeding females, numbers of fry produced, and the numbers of suitable and total shells did not differ between high- and low-density nests (Table A1). Overall, significantly more breeding females were found in high-density than in low-density nests (Table A1). Separated for moon phases, the numbers of breeding and visiting females differed between 'high-density nests' and 'low-density nests' (Table A2, Fig. A1).

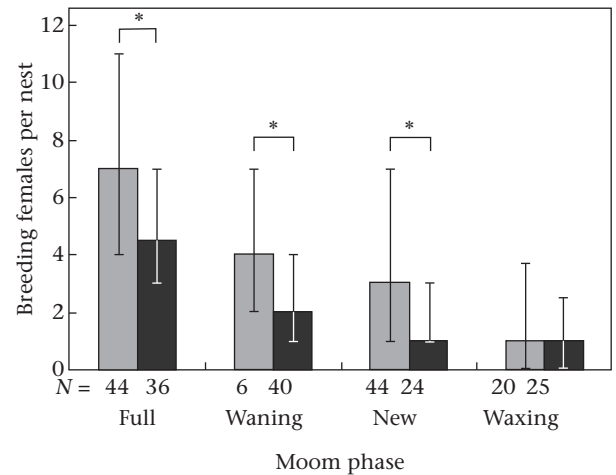


Fig. A1. Numbers of breeding females in high-density nests (grey bars) and low-density nests (black bars) at different moon phases. Medians and interquartile ranges are shown. The numbers of checked nests are indicated below the bars. Asterisks indicate a statistically significant ($P < 0.05$) difference between high- and low-density nests (see Table A2 for statistical details).