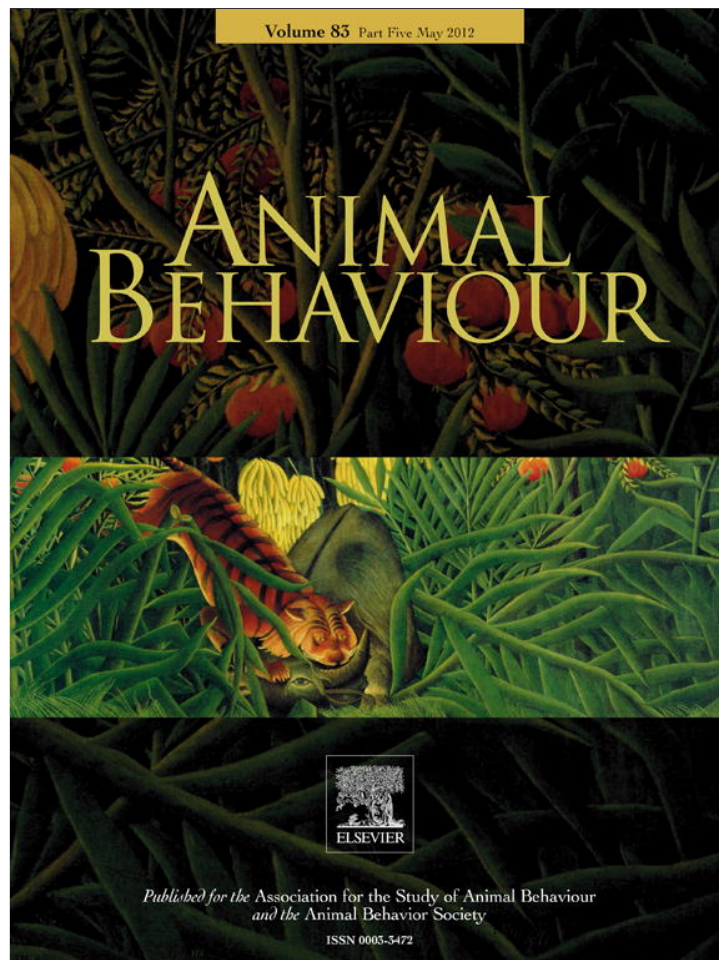


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at SciVerse ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Sex differences in the function of pair bonding in the monogamous convict cichlid

Joseph M. Leese*

Department of Biological Sciences, Lehigh University, Bethlehem, U.S.A.

ARTICLE INFO

Article history:

Received 28 October 2011
 Initial acceptance 7 December 2011
 Final acceptance 13 January 2012
 Available online 15 March 2012
 MS. number: A11-00875R

Keywords:

Amatitlania nigrofasciata
 convict cichlid
 courtship
 mate choice
 mating system
 monogamy
 pair bond
 sexual selection

Many monogamous animals engage in a period of pair bonding prior to their ultimate mate choice (reproduction). One hypothesis about the function of this behaviour is that it allows individuals to continuously assess the quality of their partner as well as other potential mates. I tested this hypothesis in the convict cichlid, *Amatitlania nigrofasciata*, by providing males and females with a choice between their partner and a novel individual of the opposite sex at various time points after formation of a pair bond. Sex differences were observed in patterns of behaviour, with females significantly more likely to spend time with their partner than with another male, while males generally spent more time with a novel female than with their partner. The time spent in a pair bond affected this pattern, however, with both sexes showing a decreased time-based partner preference as the time in a pair bond increased. After forming a pair bond, males were largely aggressive towards novel females, treating them as territorial intruders, while pair-bonded females generally ignored novel males. Neither sex appeared to actively court potential mates apart from their partner. In a second experiment, I attempted to alter this pattern by presenting pair-bonded individuals with novel potential mates of relatively higher quality (larger size) than their partner. When given a 'better option', neither females nor males showed a preference for their partner over a large novel mate. All males continued to treat novel females as territorial intruders, while a subset of females shifted their preference from their partner to a larger novel male. These results support the hypothesis that the role of pair bonding may differ between the sexes in this species, functioning as a prolonged period of mate assessment in females, but not in males.

© 2012 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

The term 'pair bond' is the common nomenclature used to describe the selective affiliation that some individuals show towards members of the opposite sex within monogamous mating systems (Lack 1940). Initially used by ethologists and applied exclusively to birds, this term has since been adopted by the fields of behavioural ecology and sociobiology to describe associations across a wide range of taxonomic groups, including humans. The formation and maintenance of a pair bond is generally thought of as the defining characteristic of monogamous species and is marked by the display of various types of mutual behaviour between partners (Lack 1940; Orians 1969; Kleiman 1977; Wittenberger & Tilson 1980; Wickler & Seibt 1983). These suites of behaviour occur for varying lengths of time prior to mating and often continue into stages of parental care.

Our current understanding of the behaviours associated with monogamy stems mostly from studies on birds where monogamy is prevalent; approximately 90% of species form pair bonds prior to copulation (Clutton-Brock 1991). The specific behaviours that these species show during pair bond formation vary, but generally

include mutual defence of a territory, construction of a nest and ritualized displays of courtship (reviewed in Birkhead et al. 1987). Apart from birds, however, monogamy is relatively rare and only occurs in 3–5% of mammals and fish (Kleiman 1977; Dunbar 1984; Gross & Sargent 1985; Clutton-Brock 1991). It is less frequent among the remaining vertebrate taxa and virtually nonexistent among invertebrates. When pair bonds form in these other taxa, the behaviours of males and females reflect those observed in birds. Territories are often secured and defended, nests are constructed and partners engage in ritualized courtship.

Despite the surfeit of descriptions of behaviours associated with pair bond formation and maintenance, evolutionary explanations for this process remain poorly understood (Kleiman 1977; Wittenberger & Tilson 1980; Reynolds 1996; Wachtmeister & Enquist 2000). Often, the display of a pair bond continues into stages of parental care and is rationalized by the need for biparental care to successfully rear offspring where a single parent could not (Clutton-Brock 1991). In some instances, however, specific behaviours occurring within a pair bond prior to fertilization remain enigmatic when they do not appear to contribute directly to offspring care. Multiple hypotheses have been provided explaining the biological significance of these behaviours, but rarely have these hypotheses been tested experimentally.

* Correspondence: J. M. Leese, Department of Biological Sciences, Lehigh University, Iacocca Hall, 111 Research Drive, Bethlehem, PA 18015, U.S.A.
 E-mail address: jml206@lehigh.edu.

The most simplistic explanation for the evolution of these behaviours is that they indeed increase fitness through contribution to parental care, albeit indirectly. The display of mutual behaviours prior to mating could help pairs coordinate their activities in preparation for shared duties required after young are born. If supported, this hypothesis predicts that pairs showing greater displays of courtship behaviour or a longer duration of time in a pair bond would be superior parents and have higher relative fitness. Griggio & Hoi (2011) showed support for this hypothesis in the bearded reedling, *Panurus biarmicus*, where pairs that engaged in long-term pair bonds had more successful breeding attempts and higher rates of successful hatchings than pairs with an abbreviated pair bond period. The benefits of increased coordination have also been demonstrated in other monogamous animals that rear subsequent broods with the same partner (Cooke et al. 1981; Choudhury & Black 1994).

A second hypothesis is that the formation of a pair bond is necessary for the synchronization of reproductive physiology between the sexes. All vertebrate taxa show a conservation of the hypothalamic–pituitary–gonadal axis, which regulates the production of gametes. In males, this system typically operates continuously, whereas females of many species do not constantly produce mature gametes (Blaustein & Erskine 2002). For the majority of monogamous species, females do not ovulate until stimulated by specific behaviours performed by their partners (Conaway 1971). As such, the formation of a pair bond is entirely necessary for successful reproduction.

A third hypothesis concerning the ultimate function of pair bonding is that it serves as a prolonged period of mate assessment during which both sexes can gather information about the quality of their partner as well as other potential mates prior to copulation (Andersson 1994; Jennions & Petrie 1997). In this case, the formation of a pair bond would be the direct evolutionary result of the action of sexual selection and be driven by the competition between and within the sexes for increased mating success. General support for this hypothesis is found in avian systems where high incidence of extrapair copulations and divorce are observed (reviewed in Choudhury 1995). Data from the monogamous convict cichlid, *Amatitlania nigrofasciata*, also suggest that this may be the primary function of pair bond formation. Both males and females show continuous assessment of mates prior to forming pair bonds (Santangelo & Itzkowitz 2004), and pair bonds can be dissociated in certain conditions (Triefenbach & Itzkowitz 1998). Males have even demonstrated bigamy in certain artificial conditions (Keenleyside 1985).

The above hypotheses are clearly not mutually exclusive. This is perhaps why few studies have attempted to delineate between them. It is more than possible, even likely, that behaviours associated with pair bond formation function in all the ways listed above. Nevertheless, the possibility exists that the ultimate function of pair bond formation differs between monogamous species because of variation in ecological niche and life history. Furthermore, if viewed in the light of sexual selection, the formation and maintenance of a pair bond could serve different purposes between the sexes within a given species. Here, I set out to determine whether the latter hypothesis, that pair bond formation provides a prolonged period of continuous assessment of mate quality, is supported in the convict cichlid fish, *Amatitlania nigrofasciata*, and furthermore, whether this function differs between the sexes. Two experiments were performed in which males and females were permitted to form pair bonds and then provided with a choice of potential mates at varying time points after pair bond formation. In each instance, one potential mate was the partner of the focal fish and the other was a novel individual. In the first experiment, novel potential mates were size-matched to the partner of the pair-bonded

individual. In the second experiment, novel individuals were 15–20% larger than partners, as size has been shown to be an indicator of mate quality in this species (Noonan 1983; Keenleyside 1985; Nuttal & Keenleyside 1993; Beeching & Hopp 1999; Gagliardi-Seeley et al. 2008). If the hypothesis were supported, focal individuals would be expected to continuously visit and court other potential mates regardless of the time spent in a pair bond. In natural settings, mate-guarding tactics and/or intrasexual competition could mask this behaviour, so I used a choice paradigm to control these influences.

METHODS

Experimental subjects consisted of laboratory-reared individuals bred from a mix of both wild-caught and commercially available convict cichlids. All animals were housed in single-sex 473-litre tanks maintained at $25 \pm 3^\circ\text{C}$ on a 14:10 h light:dark cycle and fed commercially available food pellets (Finfish Starter, Ziegler Bros Inc., Gardners, PA, U.S.A.) ad libitum. After testing, subjects were returned to separate stock tanks to avoid pseudoreplication.

I conducted two separate experiments using the same general methodology to allow comparisons. Slight modifications were made in the methods based on the sex of the focal fish to allow for size differences reflecting those found in natural populations.

At the start of each replicate, I added a single male and female convict cichlid to a 76-litre 'home aquarium' (Fig. 1a). Males in each pair were slightly larger (~ 10 mm total length) than their female counterparts, as is typical of conditions observed in the field (Wisenden 1995). Pairs were size-matched by total length, but measures of standard length, height, width and body mass were also recorded and were correlated with total length (data not shown). A clear plastic divider was placed approximately 10.0 cm from one end of the tank creating an intruder compartment

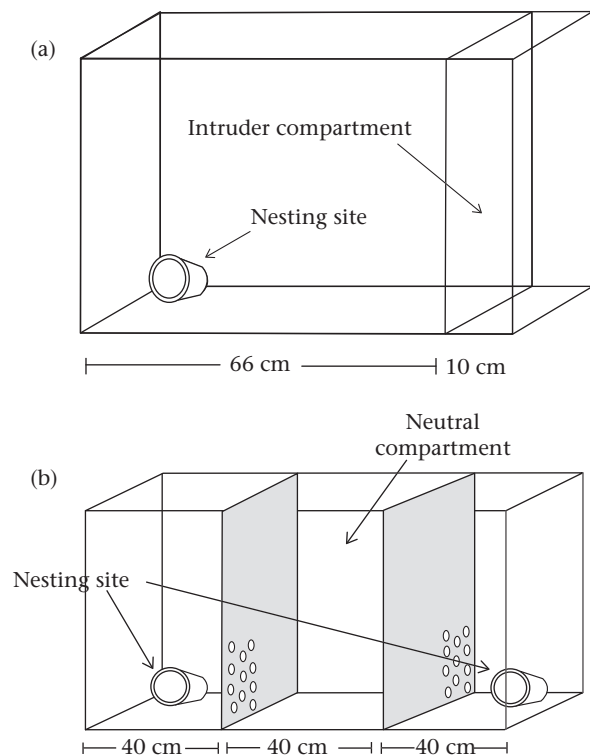


Figure 1. Experimental set-up of (a) home and (b) choice aquaria used in mate choice tests of convict cichlids. Openings in opaque dividers were offset to decrease visual contact between the outer compartments.

containing a small conspecific juvenile, which has been shown to facilitate pair bond formation in a closely related cichlid (Itzkowitz & Draud 1992). Tanks also contained an airstone and a terra-cotta flowerpot to be used as a nesting site. Home tanks were monitored daily for the formation of a pair bond, which was considered formed on the first day that individuals were observed together in a nest site or simultaneously attacking a juvenile intruder (Draud & Lynch 2002).

Once pair bond formation occurred, I dubbed a single animal within each pair the focal subject. Focal subjects were placed into one of four treatment groups associated with a specific time point after pair bond formation. These time points were unevenly spaced intervals after the first day of pair bonding and were labelled as (1) early, (2) mid, (3) late and (4) post. Subjects in the 'early' pair bond treatment group were moved to a choice aquarium on the first day of pair bond formation. Subjects in the 'mid' treatment group were moved into a choice aquarium 4 days after pair bond formation, which was considered the half-way point from pair bond formation to spawning, based on a previous study of spawning time (Bockelman & Itzkowitz 2008). The 'late' pair bonding treatment group was determined by the appearance of the female's ovipositor (Bockelman & Itzkowitz 2008; J. M. Leese, personal observation), which was used as a general indicator that a pair would soon spawn. Focal individuals in the 'late' treatment group were moved to a choice tank when the female's ovipositor descended from the cloacal opening. This occurred at an average of 10 days after the start of replicates in the male choice experiments and 11 days in the female choice experiments (supporting the use of pair day 4 as an approximate 'mid' treatment group). The final treatment group, 'post', refers to individuals tested post-spawning. In these replicates, males and females were allowed to spawn successfully in their home tank. Within 24 h after spawning, the fertilized eggs were removed from the home tank, and the focal individual was moved to a choice tank 24 h later.

At the given time point after pair bond formation, the focal individual was removed from the home aquarium and placed into a 'choice aquarium' (265 litre; Fig. 1b) containing two opaque plastic partitions creating three equal-sized compartments (similar to designs used in Santangelo & Itzkowitz 2004; Santangelo 2005; Gagliardi-Seeley et al. 2008). The partitions contained holes allowing the focal individual free access to the entire aquarium, while limiting visual contact between the outer compartments. Each of the outer two compartments contained a new flowerpot to be used as a nesting site.

Focal individuals were given 1 h to acclimate to the new aquarium, after which a potential mate was placed into each of two clear plastic box enclosures in the neutral compartment for a 4 h visual comparison period (for explanation, see Gagliardi-Seeley et al. 2008). One of the potential mates was the current partner of the focal individual, while the other was a novel fish from stock populations. In the first experiment, novel individuals were size-matched (± 3 mm) to partners, and in the second experiment, novel individuals were 15–20% larger than partners. At the conclusion of the comparison period, each potential mate was added to one of the outer compartments. The placement of individuals in outer compartments was randomized to avoid side bias and, if necessary, focal individuals were reintroduced into the neutral compartment to control for the influence of sequential mate assessment similar to that observed in sticklebacks (Bakker & Milinski 1991).

When females were focal subjects, potential male mates were too large to move through the holes in the dividers and were thus restricted to their compartments. When using males as focal subjects, it was necessary to tether potential female mates to their nest sites. Tethering was done by inserting a small microfilament

loop through the epaxial musculature of the female and attaching this loop to another piece of microfilament secured to the flowerpot. All surgical procedures were conducted in accordance with Lehigh Institutional Animal Care and Use Committee (Protocol number 86) and caused minimal distress to the animals. Tethered females could move throughout their compartment, interact with males and spawn successfully (Santangelo & Itzkowitz 2004; Santangelo 2005; J. M. Leese, personal observation).

Behavioural Observations

Behavioural observations were taken from video recordings (20 min) made approximately 24 h after introduction of potential mates using JWatcher behavioural event recorder software (Dan Blumstein's Lab, University of California Los Angeles, U.S.A.; Animal Behaviour Lab, Macquarie University, Sydney, Australia). Cichlid fishes show a suite of stereotypic behaviours described previously in various ethograms (Baerends 1986; Wisenden 1994a, b, 1995; Santangelo & Itzkowitz 2004; Oldfield & Hofmann 2011). These include, but are not limited to, bites, chases, frontal and lateral displays, brushes, tail-beats and mouth wrestling. Many of these behaviours occur in both intra- and intersexual interactions. For this study, I used a simplifying categorization with behaviours grouped as either courtship (brushes, tail-beats, quivers) or aggression (bites, chases, displays) and totalled them for two overall measurements. I also recorded the number of entries by the focal individual into each compartment as well as the focal individual's total time spent in each compartment.

Statistical Analysis

I converted the time spent in a given compartment by each focal individual to a 'strength of preference' measure by subtracting the time spent with a novel individual from the time spent with a partner. I analysed strength of preference scores with a 2×4 factorial ANOVA with focal sex (male/female) and treatment (early/mid/late/post) as independent factors. In the first experiment, significant effects of both sex and treatment prompted further analysis and post hoc comparisons were conducted within each sex using Fisher's least significant differences. Pairwise comparisons were not conducted for strength of preference measures in the second experiment because of the lack of significant overall effects.

Measures of entries into a given compartment and aggression towards potential mates did not meet the expectations of normality. I analysed the potential effects of sex and stage of pair bonding on these behaviours using a generalized linear model assuming a Poisson variance function. I conducted planned pairwise comparisons separately within each sex at each time point treatment using paired-sample Wilcoxon signed-ranks tests to determine whether focal individuals treated partners differently than novel potential mates. Observations of courtship behaviour were infrequent during the limited observation period and statistical analyses were deemed inappropriate.

RESULTS

Experiment 1: Size-matched Novel Potential Mate

Compared to focal males, focal females showed a greater time-based preference for their partner at all four stages of pair bonding (ANOVA: effect of sex: $F_{1,77} = 18.27$, $P < 0.001$; Fig. 2a). The time spent in a pair bond influenced how the sexes divided their time between their partner and novel potential mates (effect of treatment: $F_{3,71} = 4.56$, $P = 0.006$). Males showed a negative preference for their partner at most time points, indicating that they

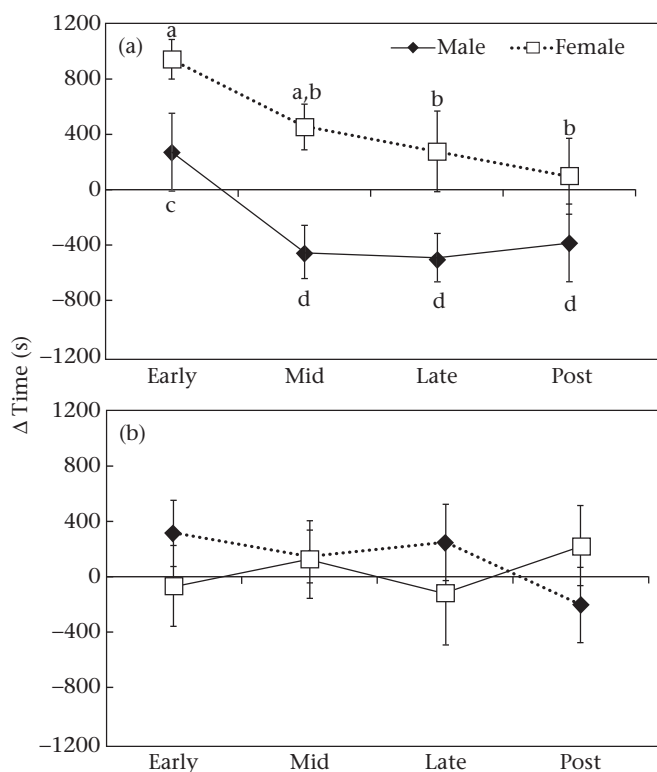


Figure 2. Mean \pm SE strength of partner preference by convict cichlids as measured by the total time spent with a partner minus the time spent with the novel potential mate when the novel individual was (a) size-matched to the partner and (b) larger than the partner. Different letters indicate significant differences between treatment groups (Fisher's LSD post hoc tests).

spent, on average, more time with the novel female than with their pair-bonded partner. The single exception occurred in the early treatment group. Females showed a positive partner preference at all stages of pair bonding, but the preference was greatest early in pair bonding, similar to the pattern observed in males. The stage of pair bonding did not influence the time that each sex spent with potential mates (interaction of sex with treatment: $F_{3,71} = 0.294$, $P = 0.83$).

Males' strength of preference for their partner was significantly greater during the early pair-bonding stage than during the mid (Fisher's least significant difference test: $P = 0.04$), late ($P = 0.03$) and post ($P = 0.05$) pair-bonding stages (Fig. 2a). The latter three groups were not significantly different from each other (mid versus late: $P = 0.90$; mid versus post: $P = 0.84$; late versus post: $P = 0.74$). Females showed a similar overall pattern; however, the preference shown early in pair bonding was not significantly different from that at mid pair bonding ($P = 0.14$), but did differ from measurements at late ($P = 0.05$) and post ($P = 0.01$) pair bonding. For females, there were no differences between the last three time points (mid versus late: $P = 0.58$; mid versus post: $P = 0.27$; late versus post: $P = 0.58$; Fig. 2a).

Males were more likely than females to enter compartments containing their partner (ANOVA: effect of sex: $F_{1,77} = 2.902$, $P = 0.004$), as well as compartments containing a novel potential mate (effect of sex: $F_{1,77} = 3.542$, $P < 0.001$). Additionally, males did not differ in the number of entries into compartments containing their partner or a novel female, regardless of stage of pair bonding (Wilcoxon signed-ranks test: early: $T = 5.50$, $N = 10$, $P = 0.08$; mid: $T = 7.00$, $N = 9$, $P = 0.24$; late: $T = 7.50$, $N = 10$, $P = 0.13$; post: $T = 27.00$, $N = 10$, $P = 0.96$; Fig. 3a), while females did. Females entered the compartment containing their partner significantly

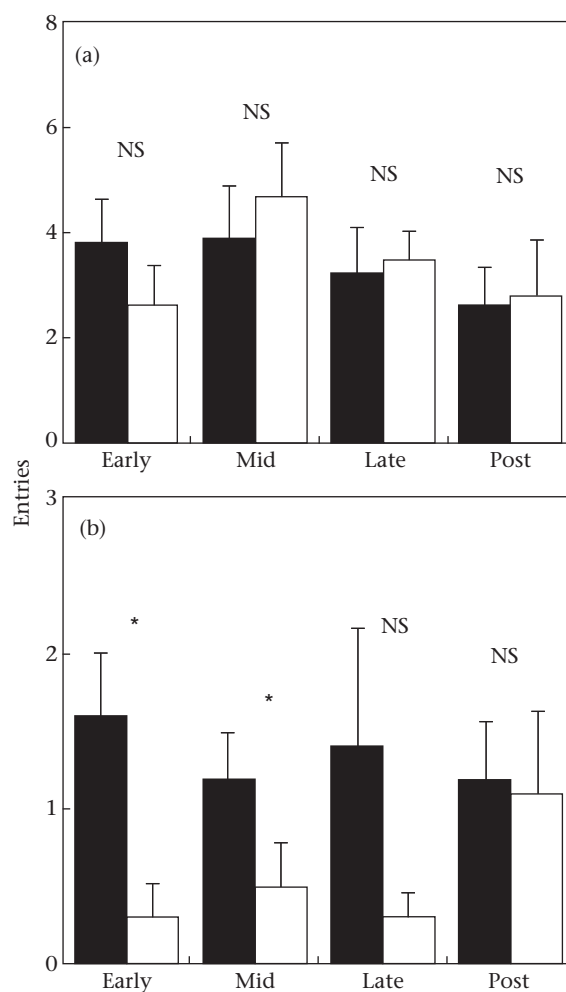


Figure 3. Mean \pm SE number of entries by focal (a) males and (b) females into compartments containing a partner (solid bars) or a novel size-matched potential mate (open bars) in experiment 1. Note difference in scales. * $P < 0.05$ (nonparametric pairwise Wilcoxon signed-ranks tests).

more often than they did the compartment containing the novel male early in pair bond formation ($T = 0.00$, $N = 10$, $P < 0.01$) as well as in the mid treatment group ($T = 0.00$, $N = 10$, $P = 0.02$). Females showed no differences in the number of entries at the late (visits: $T = 3.50$, $N = 10$, $P = 0.06$) or post (visits: $T = 10.00$, $N = 10$, $P = 0.48$) time points (Fig. 3b).

The display of stereotypic aggression and courtship behaviour were relatively sparse, with only a few individuals displaying behaviour during the 20 min video recordings. Convict cichlids are generally regarded as highly aggressive, and agonistic encounters are common both within and between the sexes. In these contests, almost without exception, the larger of two fish will win, regardless of sex (J. M. Leese, personal observation). Probably for this reason, focal females showed almost no aggression towards their partner or the novel male at any stage of pair bonding, as males were always larger and could win any contest. Focal males, conversely, were larger than both their partner and the novel female and were observed to behave aggressively towards both. Males were no more likely than females to show aggression towards partners (effect of sex: $F_{1,77} = 0.007$, $P = 0.99$), but were more aggressive than females towards novel potential mates (effect of sex: $F_{1,77} = 5.358$, $P < 0.001$). Males showed overwhelmingly more aggression towards the novel female than towards their partner at all stages of pair bonding, but this difference was only significant for the early

($T = 1.00$, $N = 10$, $P = 0.03$) and mid ($T = 0.00$, $N = 9$, $P = 0.03$) treatment groups (late: $T = 6.00$, $N = 10$, $P = 0.17$; post: $T = 3.00$, $N = 10$, $P = 0.06$). This result most likely reflects the lack of power from the use of nonparametric statistics. Females showed too little aggressive behaviour to justify statistical analysis.

Experiment 2: Large Novel Mate

When provided with a novel potential mate larger than their current partner, the sexes did not differ in their strength of preference (effect of sex: $F_{1,71} = 0.185$, $P = 0.67$) and this was independent of the stage of pair bonding (effect of treatment: $F_{3,71} = 0.082$, $P = 0.97$). Neither sex showed a preference for their partner or a large novel individual at any stage of pair bonding (one-sample t test: test value = 0; males: early: $t_9 = 1.299$, $P = 0.23$; mid: $t_8 = 0.724$, $P = 0.49$; late: $t_9 = 0.909$, $P = 0.39$; post: $t_9 = -0.745$, $P = 0.48$; females: early: $t_9 = -0.235$, $P = 0.82$; mid: $t_9 = 0.438$, $P = 0.67$; late: $t_9 = -0.318$, $P = 0.76$; post: $t_9 = 0.759$, $P = 0.47$; Fig. 2b). There was no significant interaction of focal sex with stage of pair bonding on the strength of preference ($F_{3,71} = 0.899$, $P = 0.45$). Pairwise comparisons were not conducted because of the lack of significant main effects.

As in experiment 1, males in experiment 2 were more likely than females to move between compartments, regardless of the time spent in a pair bond. Focal males entered the compartment containing their partner (ANOVA; effect of sex: $F_{1,77} = 3.507$, $P < 0.001$) and a large novel potential mate (effect of sex: $F_{1,77} = 3.885$, $P < 0.001$) significantly more often than did focal females.

In paired comparisons, focal males showed no difference in the number of entries into compartments of their partner or a novel female at any stage of pair bonding (Wilcoxon signed-ranks test: early: $T = 16.00$, $N = 10$, $P = 0.78$; mid: $T = 4.00$, $N = 9$, $P = 0.09$; late: $T = 12.00$, $N = 10$, $P = 0.39$; post: $T = 18.50$, $N = 10$, $P = 0.63$; Fig. 4a). Females also showed no significant differences in the number of entries into the compartment containing their partner and the compartment containing a novel male at any stage of pair bonding (early: $T = 14.00$, $N = 10$, $P = 1.00$; mid: $T = 14.00$, $N = 10$, $P = 1.00$; late: $T = 20.00$, $N = 10$, $P = 0.74$; post: $T = 10.50$, $N = 10$, $P = 0.14$; Fig. 4b), but the average number of entries into any compartment by focal females was less than one, suggesting little or no movement between potential mates.

The display of stereotypic aggression behaviour was again relatively sparse, with only a few individuals displaying behaviour during the limited 20 min video recordings. As in experiment 1, focal males were no more aggressive than focal females towards partners (ANOVA; effect of sex: $F_{1,77} = 0.011$, $P = 0.99$), but males were also no more aggressive than females towards large novel potential mates (effect of sex: $F_{1,77} = 0.018$, $P = 0.99$). There was only a single instance of a focal individual courting a novel potential mate, but this was most likely a result of limited observation time, as mentioned above. Males again showed, on average, more aggression towards novel females than they did towards their partner, but the difference was not significant (early: $T = 3.00$, $N = 10$, $P = 0.22$; mid: $T = 3.00$, $N = 10$, $P = 0.06$; late: $T = 2.00$, $N = 10$, $P = 0.27$; post: $T = 7.50$, $N = 10$, $P = 0.27$). Statistical analyses were not conducted on the number of aggression behaviours performed by focal females.

DISCUSSION

Both male and female convict cichlids visited novel potential mates after formation of a pair bond. The pattern of movement between potential mates differed greatly between the sexes, however, and was also affected by the time that the focal individual

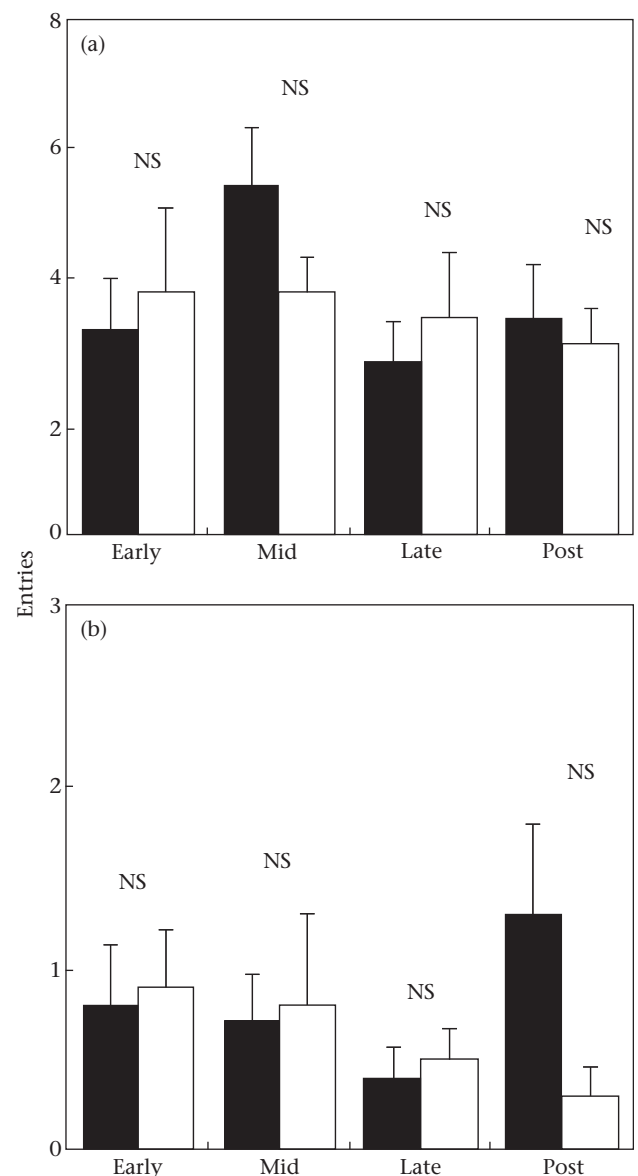


Figure 4. Mean + SE number of entries by focal (a) males and (b) females into compartments containing a partner (solid bar) or a large novel potential mate (open bar) in experiment 2. Note difference in scales. NS (nonparametric pairwise Wilcoxon signed-ranks tests).

had spent in a pair bond as well as by the relative difference in size between the partner and the novel individual.

These results are somewhat difficult to interpret in their entirety, but clear patterns emerge when the experiments are viewed independently. In experiment 1, when males were given the choice between their partner and a size-matched novel female, they showed a positive time-based partner preference early in pair bonding as would be expected in monogamy. Surprisingly, there was a reversal in their time budget activity later. Males in mid, late and post treatment groups spent significantly more time with a novel female than with their pair-bonded partner. This result could easily be interpreted as males actively pursuing mating opportunities apart from their current partner similar to extrapair copulation patterns observed in birds. Behavioural observations of aggression and courtship, however, suggest that while focal males left their partner and spent time with novel individuals, their behaviour did not reflect active mate-searching tactics. With only

a few exceptions, no courtship behaviour was performed towards novel individuals. Even though males spent more time with novel females, they would chase, bite and harass the female, often returning to the compartment containing their partner.

This behaviour is in stark contrast to studies of pair bond formation in other systems, especially monogamous mammals. Certain species of voles are used as models for monogamy, and the operational definition for the presence of a pair bond in these systems is derived from the observation of a time-based partner preference (Gavish et al. 1983). In some instances, a pair bond is not even considered formed until after mating occurs (Williams et al. 1992). This definition for the presence of a pair bond, however, might only reflect an appropriate measure for a limited number of monogamous animals that demonstrate certain ecological niches (Crews & Moore 1986). For some species, like the convict cichlid, pair bonds appear to form prior to mating and, once formed, partners often divide specific tasks and may spend little time in close proximity. For similar monogamous systems, the use of a time-based activity budget may not accurately infer the presence or absence of a pair bond.

For example, convict cichlids defend territories containing nest sites considered to be crucial to an individual's fitness (Wisenden 1995; Gumm & Itzkowitz 2007). These territories rarely contain enough food for the pair and both partners must leave to forage. Rather than abandon a nest site to forage together, one individual tends to remain and defend the territory while the other searches for food (Wisenden 1994a; Itzkowitz et al. 2001, 2002). Territorial aggression can be directed towards conspecific individuals of both sexes as either males or females could usurp a nest site or feed on unguarded young after they hatch (J. M. Leese, personal observation). In such instances, the time spent away from partners may be more indicative of territorial aggression than of active pursuit of extrapair mating attempts. Given this assumption, the observation that females tended to show a positive partner preference throughout the pair bond is logical, because if females tried to attack novel males, they would inevitably lose because of differences in relative size. High levels of aggression by males and low levels of aggression by females support this conclusion.

When both sexes were provided with a size-matched novel potential mate, focal individuals showed a stronger partner preference early in pair bond formation rather than later. It appears that proximity and possibly tactile stimulation influence the initiation of a pair bond, but once the bond is established, the need for physical proximity decreases. This could be explored on a smaller timescale of hours rather than days, to determine the point at which individuals start to show greater separation from their partner. It would also be interesting to determine whether there are sex differences at this time (i.e. whether there is a conflict between the sexes in the decision to maintain spatial proximity or to separate into sex-typical parental roles).

In experiment 2, focal individuals were given the choice between their partner and a larger novel potential mate. As is true of many vertebrate species, body size is used by convict cichlids to inform mate choice decisions. When given a choice, both males and females prefer the larger of potential mates (Noonan 1983; Keenleyside 1985; Nuttal & Keenleyside 1993; Beeching & Hopp 1999; Gagliardi-Seeley et al. 2008). These studies, however, never explored this size preference in subjects already engaged in a pair bond. In experiment 2, when individuals were provided with a choice after they were paired, neither males nor females showed a time-based preference for the larger of two potential mates. This was demonstrated at several time points after formation of a pair bond.

These results are most intriguing when compared with those of experiment 1, where clear sex differences in the strength of

preference for a given partner were observed. These sex differences again appear to reflect the different parental roles that each sex adopts after pairing, rather than a likelihood of divorce from a current partner. The results from experiment 2 complement and support that observation. Novel females in experiment 1 were similar in size to focal males, making it less likely that focal males would leave their partner to engage in territorial aggression. Both sexes of convict cichlids are highly aggressive, especially after formation of a pair bond and when providing parental care. When intersexual aggression is observed, the winner is almost exclusively the larger individual, regardless of sex (J. M. Leese, personal observation). It is possible that males may have treated novel females as intruders in experiment 2, but the novel females' large size allowed them to defend themselves, essentially forcing males to spend less time in their compartment. High levels of aggression directed towards novel females support this conclusion.

In contrast, focal females were always smaller than males in experiments 1 and 2. As such, females were unlikely to win agonistic encounters with any male, partner or novel. Their presence in the novel male compartment, then, more likely suggests a willingness to divorce a current partner for a mate of higher quality. At no time point tested did females show a mean positive preference for their partner or the large novel male.

One difficulty in interpreting the results of these experiments is the lack of behaviour observed from the video recordings. Clear displays of courtship behaviour (brushes, tail-beats and quivers) were infrequent and only observed in a few focal individuals. This finding, however, is similar to rates of courtship behaviour published elsewhere (Mackereth & Keenleyside 1993; Santangelo & Itzkowitz 2004; Santangelo 2005) and observed in natural populations of this species (J. M. Leese, personal observation). With these behaviours occurring so infrequently, they seem more likely to play a role in the continued coordination of behaviour in the pair, rather than acting as signals of mate quality. This also corroborates the finding of Santangelo & Itzkowitz (2004) that neither male nor female convict cichlids prefer individuals that demonstrate more courtship behaviours or that court at a higher rate. A second caveat to be considered is that measuring an individual's preference is difficult in monogamous systems because both sexes are expected to be choosy. The movement and behaviour of focal individuals in this choice paradigm can be influenced by the receptivity of potential mates.

While the hypothesis that pair bond formation serves as a prolonged period of mate assessment cannot be wholly rejected based on these results, I found no evidence of this function in males. Females, in contrast, may use the extended period of pair bonding to assess mates, but only when presented with a novel individual of higher quality than their partner. When females were presented with a large novel mate, some females did prefer the large individual, but their preference was apparent soon after introduction to the choice aquarium. Based on the movement data (Fig. 4b), females did not move back and forth between males and seemed to remain in a given compartment. When linked with the strength of preference data (Fig. 2b), this suggests that some females preferred their partner and others preferred the large novel male. Mate choice decisions are highly susceptible to fluctuations in environmental conditions and can change with season, predation rates, or with the preferences of other individuals in the population (Jennions & Petrie 1997). The 'lack of preference' here suggests that females vary in how and when they make decisions regarding mate choice.

In summary, these results support the notion that for most animals, mate choice decisions are more complex than previously realized, especially among monogamous animals. Clear fitness benefits have been demonstrated for both male and female convict

cichlids that choose the larger of two mates, yet the costs and benefits of mate switching after pair bond formation have not been well described. The factors determining these costs and benefits are likely to be dynamic and would need to be considered for any specific individual to predict when it might pay to abandon a current pair-bonded partner and mate with an individual of higher quality. It appears that these costs may be higher for males than for females, perhaps due to trade-offs in the parental roles that each sex adopts. The ultimate result of which is males being driven to make mate choice decisions earlier in the pair bond process than their female counterparts.

Acknowledgments

I thank three anonymous referees for their thoughtful and constructive comments of an earlier draft of this manuscript. A. Rice provided valuable statistical advice. Special thanks to M. Itzkowitz, J. L. Snekser, V. Estela and E. Becker for their assistance in the lab. This work was supported by an award from the Guy Jordan Endowment Fund of the American Cichlid Association.

References

- Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Bakker, T. C. M. & Milinski, M. 1991. Sequential female choice and the previous male effect in sticklebacks. *Behavioral Ecology and Sociobiology*, **29**, 205–210.
- Baerends, G. P. 1986. On causation and function of the pre-spawning behaviour of cichlid fish. *Journal of Fish Biology*, **29**, 107–121.
- Beeching, S. C. & Hopp, A. B. 1999. Male mate preference and size-assortative pairing in the convict cichlid. *Journal of Fish Biology*, **55**, 1001–1008.
- Birkhead, T. R., Atkin, L. & Møller, A. P. 1987. Copulation behavior of birds. *Behaviour*, **101**, 101–138.
- Blaustein, J. D. & Erskine, M. S. 2002. Feminine sexual behavior: cellular integration of hormonal and afferent information in the rodent forebrain. In: *Hormones, Brain and Behavior* (Ed. by D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach & R. T. Rubin), pp. 139–214. New York: Academic Press.
- Bockelman, A. & Itzkowitz, M. 2008. Males respond differently than females to mate loss in the biparental convict cichlid fish. *Behaviour*, **145**, 313–325.
- Choudhury, S. 1995. Divorce in birds: a review of the hypotheses. *Animal Behaviour*, **50**, 413–429.
- Choudhury, S. & Black, J. M. 1994. Barnacle geese preferentially pair with familiar associates from early life. *Animal Behaviour*, **48**, 81–88.
- Clutton-Brock, T. H. 1991. *The Evolution of Parental Care*. Princeton, New Jersey: Princeton University Press.
- Conaway, C. H. 1971. Ecological adaptation and mammalian reproduction. *Biological Reproduction*, **1**, 239–247.
- Cooke, F., Bousfield, M. A. & Sadura, A. 1981. Mate change and reproductive success in the lesser snow goose. *Condor*, **83**, 322–327.
- Crews, D. & Moore, M. C. 1986. Evolution of mechanisms controlling mating behavior. *Science*, **231**, 121–125.
- Draud, M. & Lynch, P. A. E. 2002. Asymmetric contests for breeding sites between monogamous pairs of convict cichlids (*Archocentrus nigrofasciatus*, Cichlidae): pair experience pays. *Behaviour*, **139**, 861–873.
- Dunbar, R. I. 1984. The ecology of monogamy. *New Scientist*, **103**, 12–15.
- Gagliardi-Seeley, J. L., Leese, J., Santangelo, N. & Itzkowitz, M. 2008. Mate choice in female convict cichlids (*Amatitlania nigrofasciata*) and the relationship between male size and dominance. *Journal of Ethology*, **27**, 249–254.
- Gavish, L. C., Carter, S. & Getz, L. L. 1983. Male–female interactions in prairie voles. *Animal Behaviour*, **31**, 511–517.
- Griggio, M. & Hoi, H. 2011. An experiment on the function of the long-term pair bond period in the socially monogamous bearded reedling. *Animal Behaviour*, **82**, 1329–1335.
- Gross, M. R. & Sargent, R. C. 1985. The evolution of male and female parental care in fishes. *American Zoologist*, **25**, 807–822.
- Gumm, J. M. & Itzkowitz, M. 2007. Pair-bond formation and breeding-site limitation in the convict cichlid, *Archocentrus nigrofasciatus*. *Acta Ethologica*, **10**, 29–33.
- Itzkowitz, M. & Draud, M. J. 1992. Conspecific intruders influence pair formation in a monogamous fish. *Behavioural Processes*, **28**, 59–64.
- Itzkowitz, M., Santangelo, N. & Richter, M. 2001. Parental division of labour and the shift from minimal to maximal role specializations: an examination using a biparental fish. *Animal Behaviour*, **61**, 237–245.
- Itzkowitz, M., Santangelo, N. & Richter, M. 2002. How similar is the coordination of parental roles among different pairs? An examination of a monogamous fish. *Ethology*, **108**, 727–738.
- Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, **72**, 283–327.
- Keenleyside, M. H. A. 1985. Bigamy and mate choice in the biparental cichlid fish, *Cichlasoma nigrofasciatum*. *Behavioral Ecology and Sociobiology*, **17**, 285–290.
- Kleiman, D. G. 1977. Monogamy in mammals. *Quarterly Review of Biology*, **52**, 39–69.
- Lack, D. 1940. Pair formation in birds. *Condor*, **42**, 269–286.
- Mackereth, R. W. & Keenleyside, M. H. A. 1993. Breeding territoriality and pair formation in the convict cichlid (*Cichlasoma nigrofasciatum*; Pisces, Cichlidae). *Canadian Journal of Zoology*, **71**, 960–967.
- Noonan, K. C. 1983. Female mate choice in the cichlid fish *Cichlasoma nigrofasciatum*. *Animal Behaviour*, **31**, 1005–1010.
- Nuttall, D. B. & Keenleyside, M. H. A. 1993. Mate choice by the male convict cichlid (*Cichlasoma nigrofasciatum*, Pisces: Cichlidae). *Ethology*, **95**, 247–256.
- Oldfield, R. G. & Hofmann, H. A. 2011. Neuropeptide regulation of social behavior in a monogamous cichlid fish. *Physiology & Behavior*, **102**, 296–303.
- Orians, G. H. 1969. On the evolution of mating systems in birds and mammals. *American Naturalist*, **103**, 589–603.
- Reynolds, J. D. 1996. Animal breeding systems. *Trends in Ecology & Evolution*, **11**, 68–72.
- Santangelo, N. 2005. Courtship in the monogamous convict cichlid; what are individuals saying to rejected and selected mates? *Animal Behaviour*, **69**, 143–149.
- Santangelo, N. & Itzkowitz, M. 2004. Sex differences in the mate selection process of the monogamous, biparental convict cichlid, *Archocentrus nigrofasciatus*. *Behaviour*, **141**, 1041–1059.
- Triebenbach, F. & Itzkowitz, M. 1998. Mate switching as a function of mate quality in convict cichlids, *Cichlasoma nigrofasciatum*. *Animal Behaviour*, **55**, 1263–1270.
- Wachtmeister, C. A. & Enquist, M. 2000. The evolution of courtship rituals in monogamous species. *Behavioral Ecology*, **11**, 405–410.
- Wickler, W. & Seibt, U. 1983. Monogamy: an ambiguous concept. In: *Mate Choice* (Ed. by P. Bateson), pp. 33–50. Cambridge: Cambridge University Press.
- Williams, J. R., Catania, K. C. & Carter, C. S. 1992. Development of partner preference in female prairie voles (*Microtus ochrogaster*): the role of social and sexual experience. *Hormones and Behavior*, **26**, 339–349.
- Wisenden, B. D. 1994a. Factors affecting reproductive success in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). *Canadian Journal of Zoology*, **72**, 2177–2185.
- Wisenden, B. D. 1994b. Factors affecting male mate desertion in the biparental cichlid fish (*Cichlasoma nigrofasciatum*) in Costa Rica. *Behavioral Ecology*, **5**, 439–447.
- Wisenden, B. D. 1995. Reproductive behavior of free-ranging convict cichlids, *Cichlasoma nigrofasciatum*. *Environmental Biology of Fishes*, **43**, 121–134.
- Wittenberger, J. F. & Tilson, R. L. 1980. The evolution of monogamy: hypotheses and evidence. *Annual Review of Ecology and Systematics*, **11**, 197–232.