



Female preferences for timing in a fiddler crab with synchronous courtship waving displays



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Studies of sexual communication typically focus on the design and information content of a signal of interest, but the timing of signal production relative to nearby competitors can be crucial. Male fiddler crabs, *Uca mjoebergi*, court females with a stereotyped claw-waving display, and males are often observed waving in synchrony with nearby claw-waving males. Using female mate preference experiments with robots that imitate male claw waves, we found evidence that females are more attracted to males whose waves immediately precede a synchronous group of waves (leaders); females also favoured males that waved in opposite phase to a synchronous group (alternators). By contrast, males whose waves lagged behind a group of synchronous wavers (laggards) were no more attractive. We discuss a simple sensory process that could explain how this female preference arises. Our results agree with past findings suggesting that synchrony in fiddler crabs occurs as an epiphenomenon of adaptive male responses to female preferences.

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The synchronous behaviour of many animal species has long fascinated researchers (Buck & Buck, 1968; Carlson, 1985; Gordon, 1958). Of particular interest are the synchronous calls of some anuran and arthropod species with acoustic sexual signals (Greenfield, 1994a). Generally, studies of sexual signalling focus on signal structure (e.g. Doucet, Mennill, & Hill, 2007; How, Zeil, & Hemmi, 2009; Kelley & Endler, 2012) and the information conveyed in these signals (e.g. Backwell, Christy, Telford, Jennions, & Passmore, 2000; Holman, 2012; Searcy & Nowicki, 2005). In comparison, the effect of signal timing relative to competitors on mating preferences has received little attention. But signal timing can have profound effects on mate preferences (Grafe, 1999), which can overcome preferences for other signal traits (Höbel, 2010). To date, synchronous sexual signalling has been investigated in only three channels: acoustic signals in male anurans (e.g. Greenfield & Rand, 2000) and katydids (e.g. Greenfield & Roizen, 1993), bioluminescent flashes in lampyrid beetles (e.g. Moiseff & Copeland, 2010) and claw waving by male ocypodid crabs (e.g. Backwell, Jennions, Passmore, & Christy, 1998).

Generally, synchronicity is thought to have evolved as a product of either cooperation or competition among signallers (Greenfield, 1994b). Cooperative explanations suggest that groups of synchronized males are better able to attract females than nonsynchronized

groups, thereby increasing the average fitness of synchronized males (Lloyd, 1973). This pattern could occur if synchronized, fully overlapping signals create a stronger stimulus, or if non-synchronized, partially overlapping signals create a cluttered, weak stimulus. For example, female fireflies, *Photinus carolinus*, are up to 10 times more likely to respond to synchronous (or near synchronous) simulated male flashes than nonsynchronous stimuli (Moiseff & Copeland, 2010). In this system, male synchrony also appears to maintain species/sex recognition by females against the visual clutter of other bioluminescent signals.

Synchronous sexual signalling could also evolve as an epiphenomenon of competition over signal timing among male rivals (Greenfield, 1994a). For example, in acoustic systems, overlapping signals often 'jam' one another, reducing their efficacy. Males might thus strategically alter the timing of their signals to effectively jam the signal of competitors, or to avoid being jammed by them (Greenfield, Tourtellot, & Snedden, 1997). Similarly, in some synchronously signalling species, female preferences for leading signallers that immediately precede their competitors have been demonstrated (Greenfield & Roizen, 1993; Reaney, Sims, Sims, Jennions, & Backwell, 2008). In these cases, selection should favour males that adjust their signal timing in an attempt to produce leading signals (or to avoid signalling right after a competitor), resulting in more synchronous signals being produced than expected by chance (Greenfield et al., 1997).

Cooperative and competitive explanations of synchrony are not mutually exclusive. For example, in the wolf spider, *Hygrolycosa*

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rubrofasciata, males actively synchronize their acoustic drumming sexual signals and females prefer tightly synchronized groups (Kotiaho, Alatalo, Mappes, & Parri, 2004), suggesting a cooperative origin of synchrony in this species. However, within loosely synchronized groups, females preferred leading males, whereas within tightly synchronized groups, females showed a preference for the last male to signal. Therefore, both cooperation and competition might play a role in maintaining synchronous signalling in this system.

Here, we studied female preference based on the timing of courtship waving in the synchronous signalling of the fiddler crab, *Uca mjoebergi*. Male fiddler crabs (*Uca* spp., Ocypodidae) possess one enlarged claw, which is used as a weapon during territory acquisition and defence, and is waved in a species-specific fashion to court passing females and deter rivals (How et al., 2009). In several fiddler crab species, groups of courting males wave in close synchrony (Backwell, Jennions, Wada, Murai, & Christy, 2006; Backwell et al., 1998; Gordon, 1958). In the only study experimentally addressing the adaptive function of this synchrony, Reaney et al. (2008) used mechanical imitation males to run a series of female mate preference trials in *U. mjoebergi*. They demonstrated that pairs of synchronous males had no advantage over nonsynchronous pairs, suggesting that synchrony is not a cooperative action in this system. Within nonsynchronous pairs, however, females preferentially approached the male that produced the leading wave. This supported earlier findings from the closely related *Uca annulipes* in which males visited by mate-searching females were found to produce more leading waves than their rivals (Backwell et al., 1998) probably by sneaking in extra, nonoverlapping waves as well as waving in synchrony (Backwell, Jennions, Christy, & Passmore, 1999). Taken together, this suggests that synchrony in fiddler crabs occurs as an epiphenomenon of males competing to produce leading waves.

In this study, we further investigated female preferences in *U. mjoebergi* by using robotic imitation males. By using a cluster of synchronized males and changing the timing of one focal male, we were able to assess the benefit of producing unique wave timing. Specifically, we tested whether focal males were favoured when they were either leading, lagging or waving in alternation with a synchronized group. This design allowed us to test whether the findings of Reaney et al. (2008) were driven by a leader preference or laggard avoidance, and to measure the attractiveness of males waving in alternation.

METHODS

We studied a *U. mjoebergi* population in October 2012 in East Point Reserve, Darwin, Australia (12.41°N, 130.83°E). *Uca mjoebergi* are small crabs (<20 mm carapace), living in very dense, mixed-sex populations on the intertidal mudflats of northern Australia. These crabs spend most of their lives in and around their burrows. In the mating period, receptive females leave their burrows and sequentially sample the burrows of several courting males before choosing a mate. Males court females by waving their enlarged claws. Females tend to prefer larger-clawed males, which wave faster (Kahn, Dolstra, Jennions, & Backwell, 2013; Reaney, 2009). Copulation and oviposition occur in the male's burrow, after which he leaves, with the female remaining to incubate her eggs (Crane, 1975).

We performed female mate preference experiments using custom-built robots, hereafter referred to as males. Each male consisted of a plaster replica claw (20 mm long) attached to a wire arm, which was driven by a motor buried below the claw in a plastic container. The motors were then remotely powered by a central control unit. This system was designed to perfectly imitate the waving action of male *U. mjoebergi* and has been used successfully in several past studies (e.g. Kahn et al., 2013; Reaney et al., 2008). All trials involved four males, and were run on a raised mud-covered arena (60 × 60 cm) in the field with the four males arranged in an arc 5 cm apart. The males waved continuously in a set cycle (see below) from before a female was added to the arena until shortly after the trial finished.

We performed five different treatments in our mate preference experiment (Fig. 1a). In each treatment, three of the mechanical males waved in perfect synchrony at a rate of 6.7 waves/min, with each wave taking 3 s to complete. The fourth male then waved at the same rate, but was either (1) a major leader, completing its wave as the synchronized group began theirs, (2) a minor leader, starting just before the synchronized group, half overlapping, (3) an alternator, waving in alternation with the synchronized group, (4) a major laggard, starting its wave immediately after the synchronized group finished theirs, or (5) a minor laggard, starting half way through the other waves (Fig. 1a). The position of the unique male was randomized and changed every trial, while the positions of the robotic units were randomized and changed every day.

We collected a total of 60 female mate-searching crabs for mate preference trials. Only females that were observed actively sampling signalling males were used. The females were added to the

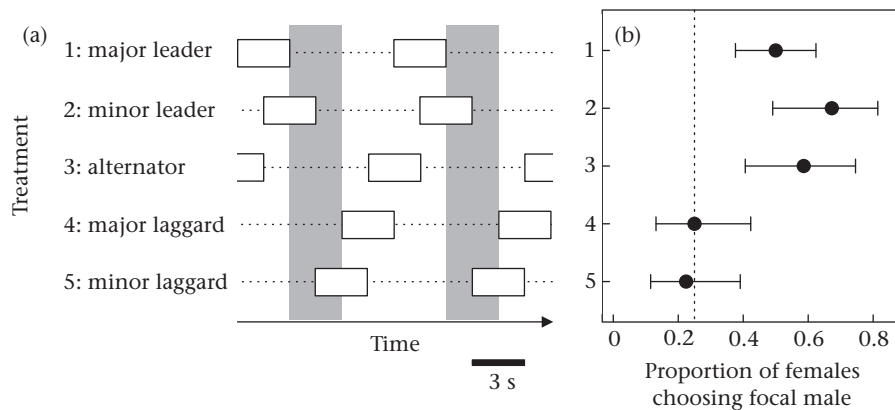


Figure 1. (a) Graphical representation of two full waving cycles for our five treatments (i.e. a snapshot of the waving seen by females). The white rectangles represent time periods in which the focal male is performing a single claw wave (3 s duration), and the shaded bars denote when the other three males are waving in synchrony. (b) Means and 95% CIs for the proportion of female crabs that chose the focal male in each of our five treatments. Shown values are from our generalized linear model. The dashed line represents the proportion of females expected by chance to choose the focal male, under the null hypothesis that all males are equally attractive.

arena in a transparent cup 20 cm from the imitation males at a random point in the waving cycle. They were then remotely released two full waving cycles after their addition. Trials ended when females either (1) made a choice (movement directly towards, then stopping at, the base of a male), (2) displayed a stereotyped startle response, e.g. in response to movement of the release cup, (3) touched the edge of the arena, or (4) did not choose a male within 3 min. Females that did not choose (outcomes 2–4) were retested a maximum of two times. Females that still did not make a choice had no data recorded for that treatment group. Each of the 60 females was exposed to each of the five treatments, and in total we recorded 287 successful trials (successful trials per treatment were 60, 55, 58, 56, 58, respectively). The order in which females experienced the treatments was randomized.

To test for differences in the proportion of females preferring the focal male across our treatments, we produced a generalized linear mixed model with binomial error structure and a logit link function. Female identity was included as a random factor to account for the fact that females were used across multiple treatments. Treatment was included as a fixed factor with treatment 1 (major leader) as the reference level. To test for an overall effect of our treatments we performed a likelihood ratio test comparing our full model to an intercept-only model. Differences between treatments were investigated using Wald's tests and confidence intervals of regression coefficients (β). Statistical analyses were performed using the lme4 package (Bates, Maechler, Bolker, & Walker, 2014) in R version 3.1.0.

Ethical Note

We captured mate-searching females by blocking the entrance to the burrow into which they were attempting to flee, then collecting them by hand. If the burrow entrance was damaged, we repaired it, leaving the burrow owner undisturbed. The female was placed in a container with a small amount of sea water (ca. 50 ml) to prevent dehydration, and kept in the shade. Each female was used in five mate preference treatments approximately 20 min apart, after which they were released. Under natural conditions, females visit up to 20 males before selecting a mate, and repeated testing of a female did not appear to stress or alter female behaviour in any way. Released females continued to mate search as normal and no female mortality occurred during this study.

RESULTS

Overall, the proportion of females choosing the focal male varied significantly across our treatments ($\chi^2_4 = 38.4$, $P < 0.0001$; Fig. 1b). This was mostly driven by a clear difference in the proportion of females visiting the focal male in the laggard (4 and 5) versus other treatments (1, 2 and 3; Fig. 1b). In both laggard treatments, females chose the focal male significantly less often than in the major leader treatment (Table 1). In both laggard

treatments the focal male was chosen at around the rate expected by chance (i.e. 1 in 4; treatment 4: 14/56 trials; 5: 13/58 trials; Fig. 1b). Exactly half the females chose the focal male in the major leader treatment (30/60 trials; i.e. more than expected by chance, which was 1/4 trials since there were four males available to be chosen). There was a nonsignificant trend for more females to choose the focal male in the minor versus major leader treatments (37/55 trials; Table 1). Finally, the focal male in the alternator treatment was chosen at a rate intermediate to the two leader treatments (34/58 trials; Fig. 1b), not significantly more often than in the major leader treatment (Table 1).

DISCUSSION

The effect of the timing of signal production on receiver preferences is relatively poorly understood. Here, we have demonstrated female preference for males that produce leading courtship waves in the fiddler crab *U. mjoebergi*, corroborating previous studies suggesting that male synchrony occurs as an epiphenomenon of female preferences in fiddler crabs (Backwell et al., 1998; Reaney et al., 2008). Our results show that this pattern is driven by a preference for leading males rather than an avoidance of laggards: leading males were preferentially chosen whereas laggards were chosen just as often as the synchronized males they followed. This suggests that there is not a direct cost to producing waves that lag behind a group of synchronized waves (females could still detect laggards, and appeared to choose them just as frequently as males in the synchronous group). We also found a (nonsignificant) trend for minor leaders (with half their wave overlapping with a synchronized group) being chosen more often than major leaders (which finished their wave as the synchronized group started). Reaney et al. (2008) did not find this pattern, although they did use a different experimental set-up. Further experiments, perhaps with varying degrees of overlap, are required to assess whether this effect is real.

A novel result of our experiment was the apparent heightened attractiveness of males waving in complete alternation with a synchronized group. What feature of leading and alternating waves makes them more attractive to females? It is not simply a matter of standing out from the group with distinct wave timing; we expected the focal male in our laggard treatments to be just as clearly distinct as in the other treatments, yet it held no extra appeal to females. We believe that the leading and alternator males had an advantage because females prefer waves that are (1) immediately preceded by a period of no waving and (2) have unique start points (i.e. only one male initiates a wave at that time). This could explain why the focal male in the laggard treatments did not appear to be disfavoured; in the laggard treatments neither the focal nor the synchronized males produced waves that satisfied both the abovementioned conditions. The start of a wave might be particularly important because this is when the claw first crosses the visual horizon of females (How et al., 2009), potentially exploiting female responses to elevated stimuli originally associated with predator response (Layne, Land, & Zeil, 1997). Taken together, our results suggest that males should avoid waving whenever they have just seen another male wave, instead of waiting until there has been a pause in the waving. If they wave then, they will produce a wave with a unique starting time. However, if all males adopt this strategy, it seems likely that multiple males will choose to wave at the same time, causing the observed synchronization of waves (Greenfield et al., 1997).

Why do females prefer waves with unique starting times, preceded by periods of 'silence'? One possibility is that the preference

Table 1
Summary of generalized linear mixed model of the proportion of females preferring the focal male in our five treatments (1: major leader used as reference category) with Wald's tests

	β	Z	P
Intercept (1: major leader)	0.00	0.00	1.00
2: minor leader	0.72	1.87	0.062
3: alternator	0.35	0.94	0.35
4: major laggard	-1.10	-2.73	0.006
5: minor laggard	-1.24	-3.05	0.002

is adaptive (as has been suggested in anurans: Richardson, Lena, Joly, & Lengagne, 2008; and insects: Party, Brunel-Pons, & Greenfield, 2014). To test for this, further experiments are required to look at which males actually produce leading and alternating waves in the field and test for correlations with other traits that could be associated with male 'quality'. Reaney et al. (2008) suggested that leadership preference in fiddler crabs may be driven by a sensory bias: a visual equivalent of the psychoacoustic 'precedence effect', in which closely overlapping sounds are perceived as a single auditory sound image, but the perceived location is assigned to the leading sound (reviewed in Litovsky, Colburn, Yost, & Guzman, 1999). This phenomenon has been demonstrated in several animals (e.g. Bosch & Márquez, 2002; Dent & Dooling, 2004) and seems to drive leadership preferences in the katydid, *Ephippiger ephippiger*, courtship call system (Greenfield, Siegfried, & Snedden, 2004), but it is unclear whether a similar effect applies to visual systems. It is important, at this point, to note that adaptive explanations and those based on sensory biases are not mutually exclusive; a sensory bias might arise adaptively (Ryan & Keddy-Hector, 1992).

Here we suggest an explanation for how the observed female preferences arise based on a simple system of signal processing. (1) When a female detects the start of a male's wave, movement is triggered towards that male. This is supported by the observation that, during mate preference trials, females usually only advance towards one of the imitation males while it is waving, and freeze between waves (personal observation). (2) But, if females receive a cluttered signal because other males wave either beforehand or at the same time, then females defer to another level of processing for comparing the males (e.g. according to claw length; Kahn et al., 2013). This arrangement of processing systems is supported by the finding that the leadership effect seems to outweigh preferences based on claw length (Backwell, n.d.). Carefully designed mate preference trials that vary the number of males and wave timing, while tracking the timing, distance and direction of female movements, could be invaluable for testing this idea.

If the female preferences based on male wave timing (and hence male synchrony) we observe in *U. mjobergi* are indeed driven by simple female sensory processes, do they exist in other species of fiddler crabs? One might expect that such processes and sensory biases would be relatively conserved among closely related taxa. For example, across most taxa females prefer signals of greater quantity (e.g. larger claws or faster waving) because they elicit stronger sensory stimulation (Ryan & Keddy-Hector, 1992). If these preferences are found across other fiddler crabs, then why don't all male fiddlers exhibit synchronous signalling? For species with very complex but variable waving patterns, and those that live in a more visually cluttered environment, males might not be able to respond to the wave timing of competitors. Similarly, perhaps the pathway from female leader/alternator preferences in fiddler crabs is mediated by population density. The species in which synchronous waving has been demonstrated (*U. mjobergi*: Reaney et al., 2008; *U. annulipes* Backwell et al., 1998; *Uca saltitanta* and *Uca perplexa*: Backwell et al., 2006) are relatively small and live in high-density, mostly single-species populations. Perhaps larger, more sparsely populated species also avoid waving during or immediately after others, but because there are fewer immediate competitors, synchrony does not arise. In fact, it may be that perfect alternation of waving arises in larger species as an epiphenomenon of the same female preferences that appear to lead to synchrony in others (Greenfield, 1994a). Studies of female preferences in nonsynchronous species would be illuminating.

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