



## Female active sampling of male paint on bowers predicts female uncertainty in mate choice



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### ARTICLE INFO

#### Article history:

Received 5 October 2015  
Initial acceptance 19 November 2015  
Final acceptance 11 February 2016  
Available online 29 April 2016  
MS. number: A15-00848R

#### Keywords:

active sampling  
mate assessment  
mate choice  
mate sampling  
mate search  
multiple mating  
*Ptilonorhynchus violaceus*  
satin bowerbird  
sexual selection  
uncertainty

It can be difficult to assess the degree to which a female attends to an individual element of a multi-component male courtship display. Quantifying behaviours where females actively sample a male display element (such as smelling or tasting a chemical signal) can provide detailed information about differences between females in their sampling behaviour and reliance on that element in making mate choice decisions. Bower 'paint' is a unique male sexual display trait found in satin bowerbirds, *Ptilonorhynchus violaceus*. Male bowerbirds masticate dried hoop pine (*Araucaria cunninghamii*) needles and apply this paste to the inside walls of their bowers. Female bowerbirds move among bowers of different males until they choose a mate. Recent mate searching experience and other factors appear to affect this process. Females visiting bowers taste the paint when they nip at the painted wall. Why females sample paint is unknown, but there is now strong evidence that female sampling of paint is important in mate selection. We tested the hypothesis that tasting is related to female uncertainty in mate choice. We found that a greater tendency of females to taste paint on bowers was associated with three measures of female mate choice uncertainty: (1) more frequent female visits to bowers, (2) mating with multiple males and (3) switching frequently among visited males. We suggest that high rates of tasting are predictive of female uncertainty in mate choice, perhaps due to some females initially having limited information about the quality of potential mates.

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Mate searching and assessment are often complicated processes in species where males have multi-element sexual displays. Courted females are exposed to different display elements either simultaneously, sequentially, or both, as they receive displays while progressing through the mate selection process. In many species, females passively observe or listen to display elements as they are courted. It is difficult to know to what degree individual females attend to all or a subset of the elements of a male's display and how they are used to make mating decisions. Several studies indicate that females may be selective in their use of information from particular display elements. For example, [Hebets and Uetz \(1999\)](#) found that females of different spider species respond

differentially to the individual components of male multimodal displays. [Coleman, Patricelli, and Borgia \(2004\)](#) showed that older females use different male display elements than younger females. [Yorzinski, Patricelli, Babcock, Pearson, and Platt \(2013\)](#) showed that, during courtship, female peafowl direct most of their attention at or below the male's head rather than focusing on the intricate and elaborated feathers of the train.

Measuring female behaviours corresponding to 'active' sampling of a male display element could provide detailed information on the degree to which females attend to, or even 'resample', a particular element and to what extent different females vary in the attention given to that element. Active female sampling behaviours can be quantified, which is a challenge to do for passive behaviours such as observing (but see [Yorzinski et al., 2013](#)) or listening. Quantifying active female sampling of individual male display traits during female mate assessment could provide insight into both the female's 'mental state' and her need to use a particular display trait in her mate choice. For example, the level of active sampling could indicate a female's level of interest in a potential mate while also helping her resolve whether to mate with him. Therefore, quantifying female active sampling of display traits could be exceedingly valuable in assessing the function of

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different male display elements, how females assess these elements and how differences among females account for observed differences in mating behaviours. Female active sampling behaviour may indicate individual variation in the pattern of decision making and reveal differences in how individual females use particular male display elements.

Active sampling of male traits seems commonly related to chemical traits such as olfactory or gustatory cues. In mammals, sniffing by females of male faeces, urine or glandular fluids can affect mate choice and fetal development (Singer, Beauchamp, & Yamazaki, 1997). Female *Drosophila* touch males to sample cuticular pheromones (Howard, Jackson, Banse, & Blows, 2003) as do female crickets (Thomas & Simmons, 2009), female beetles (Peterson, et al., 2007) and females in other insects. Chemical signals are now recognized as important in avian communication (Hagelin & Jones, 2007; Whittaker, Gerlach, Soini, Novotny, & Ketterson, 2013). Chemosensory signals are part of the complex, multimodal displays of crested auklets, *Aethia cristatella* (Hagelin, 2007; Hagelin, Jones, & Rasmussen, 2003). Bird odour is predictive of reproductive success in a common passerine, dark-eyed juncos, *Junco hyemalis* (Whittaker et al., 2013). In addition, chemosensory signals are suspected in the 'exploded lek' system of the endangered kakapo, *Strigops habroptilus* (Gsell, 2012; Hagelin, 2004). However, chemical display traits have not been identified in other lekking birds apart from 'paint' applied to bowers by male satin bowerbirds, *Ptilonorhynchus violaceus*, and tasted by mate searching females (Bravery, Nicholls, & Goldizen, 2006; Hicks, Larned, & Borgia, 2013; Robson, Goldizen, & Green, 2005).

In addition to active sampling of male display traits, female signals to males in response to male displays may also reveal females' mental states. Signals differ from active display trait sampling because they function to influence the behaviour of the displaying male. For example, female cowbirds produce wing strokes in response to males singing attractive songs. This stimulates males to sing more of these effective songs, triggering female copulatory postures (Gros-Louis, White, King, & West, 2003; King, West, & White, 2003; West & King, 1988). Female wolf spiders have receptive postures that have been used to demonstrate preferences for particular components of male displays (Hebets & Uetz, 1999). Females also often signal readiness to mate (e.g. Demary, Michaelidis, & Lewis, 2006; Patricelli, Uy, & Borgia, 2004; Patricelli, Uy, Walsh, & Borgia, 2002; Santangelo, 2005).

Satin bowerbird males have displays involving multiple elements that include the bower, bower decorations and coordinated visual and vocal displays, which are assessed by females in multiple visits to bowers (Borgia, 1985; Patricelli et al., 2002; Robson et al., 2005; Uy, Patricelli, & Borgia, 2000). Studies of satin bowerbirds have demonstrated the value of quantifying variable female behaviours during courtship that reveal a female's mental state. Males court females at their bowers with energetic dancing and vocal displays (Coleman, Patricelli, Coyle, Siani, & Borgia, 2007; Loffredo & Borgia, 1986). A female visiting a bower typically starts in a standing position and then after several visits, lowers into a crouch, indicating her comfort with the male and his display, and finally lifts her tail to signal her readiness to copulate (Patricelli et al., 2002). Female age affects display preferences, with older females preferring intense male courtship displays and young females attending more to blue decorations (Coleman et al., 2004). Young females appear to be threatened by intense courtships, which often cause them to startle out of a crouch position and sometimes leave the bower (Coleman et al., 2004; Patricelli et al., 2004). These studies of crouching signals have allowed us to consider how female behaviour in the bower can indicate a female's reaction to male displays and her readiness to mate. Even with this information, however, it can be difficult to determine to what degree and

when in the mate selection process a female is focusing on a particular aspect of male display because crouching is likely influenced by multiple elements of the male display and also occurs relatively late in the mate searching process (Patricelli et al., 2004).

The paint applied to bowers by male satin bowerbirds is a trait used by females in mate assessment (Hicks et al., 2013). Throughout the mating season, male satin bowerbirds chew dried hoop pine (*Araucaria cunninghamii*) needles and apply the resulting brown paste on the inside of their bower walls, focusing on the area midway up the walls at beak level. Bravery et al. (2006) found that males painted for 24% of the total amount of time they spent at their bowers, roughly equal to the time spent on bower building. In addition, Robson et al. (2005) found that male painting rate is correlated with mating success. During visits to bowers, females nip at the bower walls and swallow, appearing to taste the paint (Bravery et al., 2006; Hicks et al., 2013; Robson et al., 2005). Such tasting occurs at 39% of all courtship visits (Bravery et al., 2006; Robson et al., 2005), suggesting that paint may be a chemical signal used in mate assessment. More specifically, Reynolds et al. (2009) speculated a role for paint in kinship recognition. Hicks et al. (2013) showed that females' revisits to bowers are reduced by paint removal.

Studying female bowerbird sampling of paint offers a potentially important and novel window into female assessment of males. While crouching by a female acts as a signal indicating the female's comfort and her readiness to mate (Patricelli et al., 2004, 2002), tasting could provide information about how a specific display element, paint, may affect female decision making during mate searching. In addition, tasting behaviour can occur at any point in the mate searching process, including early during mate searching, or even when the male is absent from his bower. By studying this behaviour, we can examine how tasting relates to mate assessment. We can measure (1) whether and when females taste during mate searching, (2) whether individual female differences relate to differences in tasting behaviour and (3) whether individual differences in tasting behaviour provide insight into a female's mental state during her search.

Female mate searching can be difficult to quantify. However, in the Wallaby Creek bowerbird population (New South Wales, Australia), we have been able to uniquely band most females and follow them through the mate searching process using video cameras positioned at bowers that automatically turn on when birds arrive. In the first detailed examination of female satin bowerbird mate searching, Uy, Patricelli, and Borgia (2001a, 2001b) showed that most females visit a sample of the males in the population, which they narrow down across subsequent visits. Females vary in the initial number of males they visit, total number of visits and the speed at which they conclude their mate search (Uy et al., 2001a). After multiple visits to males, most females (84%) choose a single male for a mate (Reynolds et al., 2009). Although this suggests that females become more certain about their mate choice as they progress towards mating, the pattern of this narrowing process varies among females. A subset of singly mating females, referred to as 'faithful' females, mate with the same male as the previous year, with less searching than other females that switch mates between years (Uy et al., 2000, 2001a). This suggests that females who switch mates, referred to as 'unfaithful' females (Uy et al., 2000), are less certain of who their mate will be than faithful females, at least in early stages of mate selection.

Here we relate female differences in tasting behaviour to differences among females in their mate searching and mating behaviour. During a preliminary study of female tasting behaviour, we examined this behaviour across various visit types at bowers: visits without courtships, courtships, and courtships ending in copulation. We found significant differences in tasting behaviour

and that the proportion of visits with tastes was lowest during courtships ending in copulation. This preliminary result suggested that females might use paint tasting for mate assessment more during the early part of the mate selection process. This led to the hypothesis that females who tasted in a greater proportion of their mate searching visits were less certain in their mate choice.

Although female uncertainty in mate choice is difficult to measure directly, there are several variables that are indicative of uncertainty in mate selection that we were able to use to test the hypothesis that uncertain females utilize tasting more. First, we quantified the number of visits and number of males visited by females, predicting that females that make more visits and visit more males will have a greater proportion of visits with tastes. Second, based on mating patterns, we assigned females to one of three mating classes that likely reflect increasing premating uncertainty in mate choice: females faithful to the previous year's mate, females that mated with a single male but not the same male from the previous year, and females that mated with multiple males. We predicted that faithful females would taste the least, those switching mates would taste an intermediate amount, and the seemingly least certain multiple maters would taste the most. Third, we quantified the relationship between tasting and switching among bowers visited, assuming that frequently alternating between different males indicates uncertainty by searching females. We predicted that switching would be positively related to the proportion of visits with tastes. If number of visits, number of males visited, female mating class and switching among males are all associated with tasting, it would suggest that tasting is used to gain additional information when females are uncertain in mate choice. In addition, we examined the relationship between age and tasting, since tasting and uncertainty may be related to female experience, and age effects have been found in female assessment of other display elements (Coleman et al., 2004).

## METHODS

### *Behavioural Monitoring*

This study was conducted in Wallaby Creek, New South Wales, Australia (28°28'S, 152°30'E). Male and female satin bowerbirds were uniquely banded before the mating season (Borgia, 1985). This bowerbird population has been banded from 1977 to 1987 and then continuously since 1995. From our banding records, we were able to determine the minimum possible age for each female observed in this study. Bowers were monitored with Hi-8 video cameras controlled by an infrared sensor that recorded all breeding season behaviours (Borgia, 1995). Data were collected at 37 bowers in 2003 and 34 bowers in 2004. This included all bowers found by intensive searching throughout the study site. Female mate searching patterns were reconstructed from date and time stamps on the videotapes with 2004 as the focal year.

Female visits at bowers were separated into three types: 'non-courtship' visits when no bower owner was present ( $N = 212$ ), 'courtship' ( $N = 495$ ), or 'copulation' when a courtship ended with mating ( $N = 208$ ). Female tastes during any of these visits were scored as 'yes' or 'no' for the visit.

### *Female Mating Classes*

To examine how female mating class influences tasting behaviour, we defined three exclusive categories: (1) 'faithful' females were those that mated with the same male in 2003 and 2004 ( $N = 13$ ; see Uy et al., 2001a); (2) 'multiple-mate' females mated with more than one male in 2004 ( $N = 14$ ; range 2–3 males, mean  $\pm$  SD =  $2.14 \pm 0.36$  males); and (3) 'novel-mate' females, an

intermediate mating class, mated with one male in 2004 that was not their mate in 2003, either because the female did not mate in 2003 or she switched mates between 2003 and 2004 ( $N = 38$ ). Females that did not fit into any of these classes ( $N = 21$ ), such as females that were not present in 2003 (i.e. 1-year-old females,  $N = 4$ ) or females that were present in 2003 but did not mate in 2004 ( $N = 14$ ), were excluded from these analyses.

Consistent with an earlier study (Uy et al., 2001a), multiple-mate females visited significantly more males than either faithful females (Mann–Whitney  $U$  test:  $U = 160.0$ ,  $N_1 = 14$ ,  $N_2 = 13$ ,  $P = 0.00064$ ) or novel-mate females ( $U = 409.5$ ,  $N_1 = 14$ ,  $N_2 = 38$ ,  $P = 0.0025$ ) and tended to appear more times at bowers (multiple-mate versus faithful:  $U = 128.0$ ,  $N_1 = 14$ ,  $N_2 = 13$ ,  $P = 0.071$ ; multiple-mate versus novel-mate:  $U = 431.5$ ,  $N_1 = 14$ ,  $N_2 = 38$ ,  $P = 0.00062$ ). To control for these differences between female groups, the proportion of visits with tastes was always used during analyses (see *Statistical Analyses*). There was no significant age difference between faithful and multiple-mate females ( $U = 85.0$ ,  $N_1 = 13$ ,  $N_2 = 14$ ,  $P = 0.77$ ). Novel-mate females were significantly younger than faithful and multiple-mate females (novel-mate versus faithful:  $U = 151.5$ ,  $N_1 = 38$ ,  $N_2 = 13$ ,  $P = 0.038$ ; novel-mate versus multiple-mate:  $U = 174.5$ ,  $N_1 = 38$ ,  $N_2 = 14$ ,  $P = 0.057$ ).

### *Statistical Analyses*

Data were analysed using R v.3.2.0 (R Core Team, 2015). To investigate how tasting behaviour varied across female visit types, we calculated for each female the proportion of each visit type where tasting occurred. This controlled for the different number of visits per female. We calculated bower switching in two ways because the total number of bower switches can be greatly affected by the total number of males that a female visits during the mating season. The first measure, the number of excess switches, was calculated as the difference between the number of times a female switched between bowers in her mate searching sequence and the minimum number of switching she must have done in order to visit all the males she observed. This measure controls for the amount of switching that necessarily occurs when visiting more than one male. The second measure of bower switching was calculated as the proportion of bower switches, which is the number of switches divided by the number of males visited.

We checked data for normality and homogeneity of variances, and these parametric test assumptions were violated. Therefore, we rank-transformed the data and used the appropriate nonparametric tests. We used Mann–Whitney  $U$  tests to determine differences between independent groups, Wilcoxon signed-ranks tests to determine differences between paired observations, and Spearman's rank correlations to test for correlations.

We predicted a priori that the proportion of visits with tastes would be positively related to behaviours suggesting less certainty in female choice of a mate, such as number of visits, number of males visited and amount of switching among bowers visited, because these females invest more in mate searching among males. All statistical tests are presented as two tailed.

### *Ethical Note*

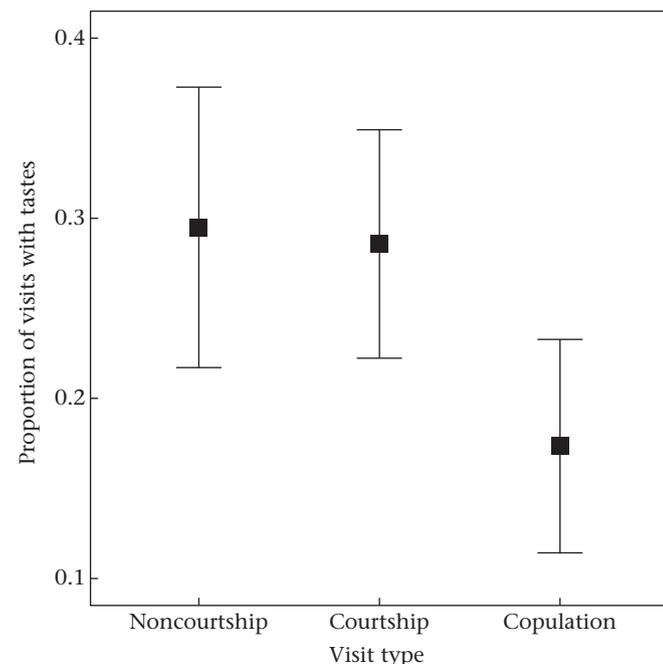
Capture, banding and experimental protocols were approved by the University of Maryland's Institutional Animal Care and Use Committee (R-04-37) and, locally, by the University of Wollongong Animal Ethics Committee (AE02/18). Research was conducted in New South Wales under New South Wales National Parks and Wildlife Services license number S10516, and birds were captured for banding under Australian Bird and Bat Banding Scheme authority numbers 2594, 2539 and 946.

## RESULTS

We confirmed our preliminary observation that less tasting occurred in courtships ending in copulations (Fig. 1). We found that tasting occurred proportionately less during visits ending in copulation than during visits without courtship (Wilcoxon signed-ranks test:  $W = 398.0$ ,  $N = 86$ ,  $P = 0.0048$ ) or visits with only courtship ( $W = 513.5$ ,  $N = 86$ ,  $P = 0.0081$ ). The proportion of visits with tastes did not differ significantly between visits where courtship occurred but did not end in copulation and visits without courtship ( $W = 1010.0$ ,  $N = 86$ ,  $P = 0.81$ ).

We examined the relationship between tasting and both the number of males visited and the total number of visits, assuming more males and more visits suggest a higher level of uncertainty in mate choice. Supporting our hypothesis, we found a trend for a positive relationship between the proportion of visits with tastes and the total number of visits at bowers (Spearman rank correlation:  $r_s = 0.18$ ,  $N = 86$ ,  $P = 0.10$ ) and a significant positive relationship between the proportion of visits with tastes and the number of males visited ( $r_s = 0.24$ ,  $N = 86$ ,  $P = 0.025$ ). There were 18 females that did not taste during their mate search. These females did not differ from tasting females by age (Mann–Whitney  $U$  test: nontasting versus tasting:  $U = 713.5$ ,  $N_1 = 18$ ,  $N_2 = 68$ ,  $P = 0.28$ ). Nontasting females visited significantly fewer males ( $U = 317.0$ ,  $N_1 = 18$ ,  $N_2 = 68$ ,  $P = 0.0013$ ) and appeared fewer times at bowers ( $U = 163.5$ ,  $N_1 = 18$ ,  $N_2 = 68$ ,  $P < 0.0001$ ) than females that tasted at least once during their mate search. Since the nontasting females searched less, this result supports our hypothesis that tasting is related to more uncertainty, as reflected by more visits during mate searching.

We next examined how tasting relates to mating class, excluding from our analyses those females that were not present in 2003 or that did not mate in 2004 (see *Methods*). Tasting females were evenly distributed among the three female mating classes: 85% of faithful females (11/13), 82% of novel-mate females (31/38) and 100% of multiple-mate females (14/14) tasted at least once during their mate search (chi-square test:  $\chi^2_2 = 2.94$ ,  $P = 0.23$ ).



**Figure 1.** Mean proportion of visits in which tasting occurred, subdivided by visit type. Error bars are 95% confidence intervals.

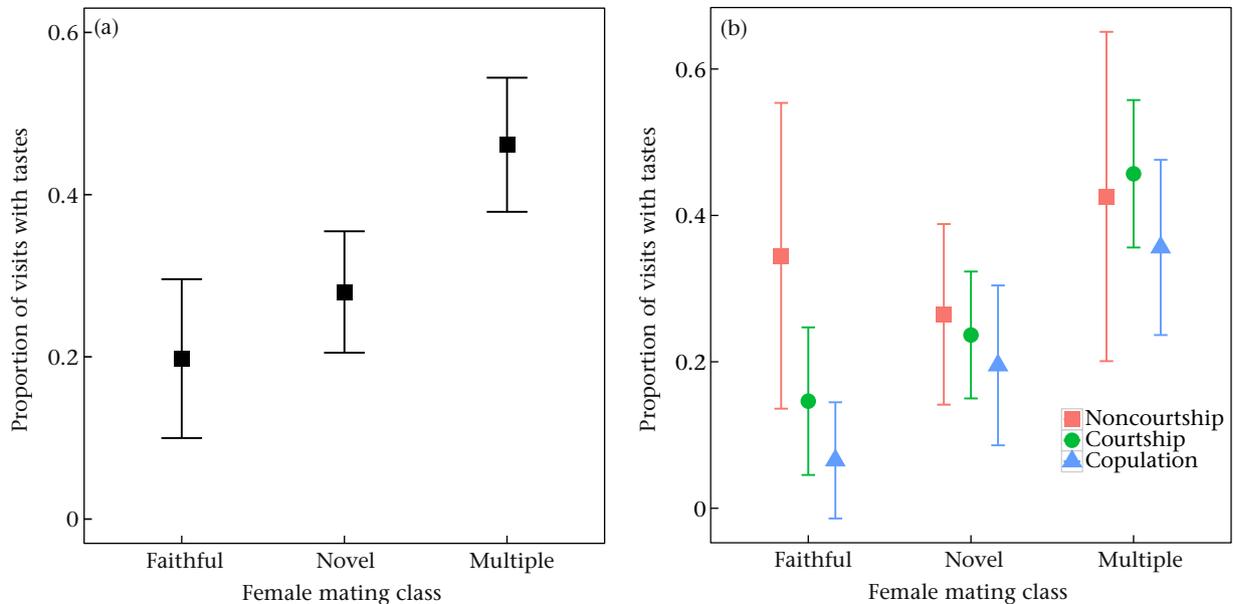
Faithful females did not differ from multiple-mate or novel-mate females in the proportion of males they tasted (Mann–Whitney  $U$  test: faithful versus multiple-mate:  $U = 86.0$ ,  $N_1 = 13$ ,  $N_2 = 14$ ,  $P = 0.80$ ; faithful versus novel-mate:  $U = 300.0$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.24$ ), although novel-mate females tended to taste a lower proportion of males than multiple-mate females ( $U = 175.0$ ,  $N_1 = 38$ ,  $N_2 = 14$ ,  $P = 0.055$ ). Nor did faithful, novel-mate and multiple-mate females differ in the probability of tasting at least once the point of the male they eventually copulated with (faithful versus multiple-mate:  $U = 90.0$ ,  $N_1 = 13$ ,  $N_2 = 14$ ,  $P = 0.96$ ; novel-mate versus multiple-mate:  $U = 210.0$ ,  $N_1 = 38$ ,  $N_2 = 14$ ,  $P = 0.19$ ; faithful versus novel-mate:  $U = 281.5$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.38$ ).

We examined how the overall proportion of visits with tastes was related to different females' mating classes (Fig. 2a). We found no significant difference between the proportion of visits with tastes between faithful and novel-mate females ( $U = 194.0$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.25$ ). However, they both had a significantly smaller proportion of visits with tastes than multiple-mate females (faithful versus multiple-mate:  $U = 19.5$ ,  $N_1 = 13$ ,  $N_2 = 14$ ,  $P = 0.00050$ ; novel-mate versus multiple-mate:  $U = 124.0$ ,  $N_1 = 38$ ,  $N_2 = 14$ ,  $P = 0.0033$ ). Overall, multiple-mating females tasted proportionately more during their mate search than singly mating females.

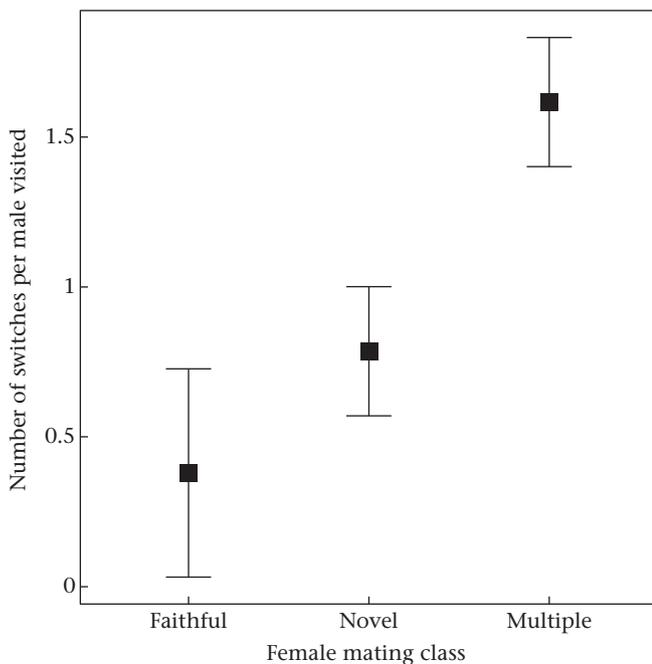
We decomposed the proportion of visits with tastes for each female mating class into the proportion of tasting per visit type (Fig. 2b). There was only one significant difference between visit types within a female mating class: faithful females tasted proportionately less during courtships ending with copulation than during noncourtship visits (Wilcoxon signed-ranks test:  $W = 1.5$ ,  $N = 13$ ,  $P = 0.013$ ). There were, however, significant differences between female mating classes. While faithful and novel-mate females did not differ significantly from each other in the proportion of tasting across any visit type (Mann–Whitney  $U$  test: noncourtship:  $U = 288.5$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.32$ ; courtship:  $U = 201.0$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.30$ ; copulation:  $U = 211.5$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.34$ ), they both differed from multiple-mate females during courtships and courtships ending with copulation. Multiple-mate females tasted proportionately more during courtships and courtships ending in copulations than faithful females (courtship:  $U = 162.5$ ,  $N_1 = 14$ ,  $N_2 = 13$ ,  $P = 0.00045$ ; copulation:  $U = 158.0$ ,  $N_1 = 14$ ,  $N_2 = 13$ ,  $P = 0.00065$ ) or novel-mate females (courtship:  $U = 423.5$ ,  $N_1 = 14$ ,  $N_2 = 38$ ,  $P = 0.00099$ ; copulation:  $U = 381.0$ ,  $N_1 = 14$ ,  $N_2 = 38$ ,  $P = 0.0096$ ).

Examining the relationship between tasting and bower switching, we found across all females that the proportion of visits with tastes was significantly correlated with the number of excess switches among bowers (Spearman rank correlation:  $r_s = 0.30$ ,  $N = 86$ ,  $P = 0.0055$ ) as well as with the number of switches per male visited ( $r_s = 0.25$ ,  $N = 86$ ,  $P = 0.02$ ). In addition, supporting our prediction, multiple-mate females had more excess switches than either faithful (Mann–Whitney  $U$  test:  $U = 170.5$ ,  $N_1 = 14$ ,  $N_2 = 13$ ,  $P < 0.0001$ ) or novel-mate ( $U = 453.5$ ,  $N_1 = 14$ ,  $N_2 = 38$ ,  $P < 0.0001$ ) females. Faithful and novel-mate females did not differ in their number of excess switches ( $U = 187.0$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.14$ ). Similar results were found when considering the number of switches per male visited, although in this case, all mating classes differed (multiple-mate versus faithful:  $U = 170.5$ ,  $N_1 = 14$ ,  $N_2 = 13$ ,  $P < 0.0001$ ; multiple-mate versus novel-mate:  $U = 455.5$ ,  $N_1 = 14$ ,  $N_2 = 38$ ,  $P < 0.0001$ ; faithful versus novel-mate:  $U = 156.5$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.044$ ; Fig. 3).

We next examined the relationship between mating class and the proportion of males rejected (mean  $\pm$  SD: faithful:  $0.25 \pm 0.36$ ; novel-mate:  $0.47 \pm 0.31$ ; multiple-mate:  $0.42 \pm 0.25$ ). Faithful females had a tendency to reject a lower proportion of males than novel-mate females ( $U = 161.5$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.054$ ), but



**Figure 2.** Mean proportion of visits in which tasting occurred, subdivided by (a) female mating class and (b) female mating class and visit type. Error bars are 95% confidence intervals.



**Figure 3.** Mean number of switches per male visited, subdivided by female mating class. Error bars are 95% confidence intervals.

this probably reflects them visiting fewer males, and often only one male ( $U = 161.0$ ,  $N_1 = 13$ ,  $N_2 = 38$ ,  $P = 0.054$ ). There were no significant differences between the other mating classes in the proportion of males they rejected (faithful versus multiple-mate:  $U = 62.5$ ,  $N_1 = 13$ ,  $N_2 = 14$ ,  $P = 0.15$ ; novel-mate versus multiple-mate:  $U = 307.5$ ,  $N_1 = 38$ ,  $N_2 = 14$ ,  $P = 0.38$ ).

Females that are uncertain of which male to choose may need to sample paint from bowers more often because they are less familiar with those males. One dramatic reason that a female may need to sample unfamiliar males is if her previous mate dies (Uy et al., 2001b). There were only three cases where a female mating in

2004 had previously mated with a male in 2003 who later died (one novel-mate female and two unclassified females). However, there are at least two other ways we can test this familiarity hypothesis. First, we can ask whether females tend to taste more at the bowers of males that they did not visit the previous year compared to those males that they did visit. Although females did taste proportionately less at bowers of males they had sampled the previous year, this difference was not statistically significant (Wilcoxon signed-ranks test:  $W = 142.0$ ,  $N = 30$ ,  $P = 0.39$ ). However, perhaps many females still taste bowers they have sampled the previous year to update their information or confirm males' identities. A different test of the familiarity hypothesis is that females should taste less as they become more familiar with a male. One prediction of this hypothesis is that females should taste more on their first visit with a male than on their second visit, which was the case ( $W = 419.5$ ,  $N = 74$ ,  $P = 0.036$ ).

When we related tasting behaviour to age, we found no significant correlation between age and the proportion of all visits with tastes (Spearman rank correlation:  $r_s = -0.15$ ,  $N = 86$ ,  $P = 0.16$ ). There was a tendency for females 20+ years old to taste less than younger females (Mann–Whitney  $U$  test:  $U = 44.5$ ,  $N_1 = 3$ ,  $N_2 = 83$ ,  $P = 0.058$ ), although with the small sample size of very old females, this may not be meaningful.

## DISCUSSION

Our results support the hypothesis that females attend to paint more when they are uncertain in mate choice. We developed this hypothesis based on earlier work by Uy et al. (2000, 2001a, 2001b), who showed that females with more information about high-quality males from their mate searching experience the previous year had shorter mate searches the following year. This result suggested that females with more useful information about potential high-quality males had higher certainty (lower uncertainty) about their choice of a future mating partner, and those females should resolve their mate searches more quickly. Females with less relevant experience should show higher levels of uncertainty leading to more sampling of males. In this paper we extend this

logic to active female sampling of a male trait, bower 'paint', at male display courts.

Bowerbirds are a particularly good system for studying active female sampling and uncertainty in mate choice for several reasons. First, display sites of male satin bowerbirds are widely separated, so there is no ambiguity in identifying the male that is being assessed by the visiting female, as compared to traditional leks, where visiting females can simultaneously be courted by several males. Second, because we could continuously monitor behaviour at bowers with video cameras, we could quantify female active sampling of paint as well as reconstruct patterns of female visits to construct our different measures of uncertainty. Lastly, because paint is applied to bower walls before courtship begins, it is accessible to females throughout mate searching and courtship, and therefore, the female controls when sampling occurs.

The hypothesis that active paint sampling during courtship by females is related to level of uncertainty was supported by four lines of evidence. First, tasting occurred more often during visits where females did not make mate choice decisions (Fig. 1). Second, we showed that the proportion of visits with tastes was positively correlated with the number of males visited and the total number of visits by females to males' bowers. Also, nontasting females visited fewer males. Third, when females were categorized into mating classes, multiple-mate females, the class of females that seemed to show the highest level of uncertainty, had a greater proportion of visits with tastes (Fig. 2a). This was especially true during courtships and during courtships ending with copulation, compared to females that appeared more certain in mate choice (i.e. singly mating females; Fig. 2b), as would be expected if females use paint to resolve uncertainty and multiple mating reflects an inability to make definitive mate choice decisions. Fourth, we found that the proportion of visits with tastes was correlated with the number of excess switches as well as with the number of switches per male visited. Multiple-mate females in particular had more switches than faithful or novel-mate females for both measures of switching (Fig. 3).

Other alternative hypotheses were less well supported. For example, some females may be less adept at assessing chemical signals, leading them to taste more. This would either predict a negative relationship between tasting and age, or if female inability to assess chemical signals cannot be improved over time, consistency in tasting behaviour between years. We do not have evidence for age-related changes in tasting (see below). While we did not have multiple years of data on tasting, female variation in mate searching behaviour across years (Uy et al., 2000, 2001b) makes consistency in tasting behaviour between years unlikely. Alternatively, females may vary in their motivation and search and taste in relation to this trait. However, faithful females that search and taste less are more likely to mate with high-quality males (Uy et al., 2000), and it is unlikely that less motivated females would be better at finding high-quality males. Another possibility is that a female might taste less when being courted by the male bower owner because she must attend to his other display traits. However, we found no difference between the tasting behaviour during visits without the male present and courtships that did not end in copulation. Tasting was only reduced during courtships that ended in copulation. During these visits, females have already completed the construction of their nests and are fertile, and it is likely that they have already made a decision to mate (Uy et al., 2001a). Finally, tasting might function as a signal to males about a female's mental state. However, given that tasting occurs whether or not males are present, the primary role for tasting cannot be as a signal in the way that crouching is a signal of female comfort with male display (e.g. Patricelli et al., 2002, 2004). None of these alternative hypotheses are compelling given the data.

According to our hypothesis that active paint sampling by females is related to uncertainty, females should become more certain and taste less as they become more familiar with males they sample. This was suggested by our finding that females tasted more during visits that did not end in copulation compared to visits where females made a final mating decision. Even stronger support for this hypothesis is our finding that females tasted less during their second visit to a particular male than on their first visit. These results support the claim that male display traits are more actively sampled by information-limited females and that sampling is reduced as uncertainty decreases. However, females also may differ in their ability to reduce uncertainty if (1) females vary in their ability to remember or compare male trait values from previous visits, (2) initially information-limited females lose track of their previous visits because they tend to search among more males, or (3) nonrandom distributions of males lead to differences between females in the range of male quality they experience. Therefore, female uncertainty in the ability to select among males may not only result from the information that females have gained from recent searches, but could also be affected by differences in their cognitive ability or the quality of the set of males they are able to visit.

Although other studies of bowerbirds have shown that females of different ages attend to decorations and courtship displays differently (Coleman et al., 2004), we did not find an effect of age on female tasting behaviour. In addition, we did not find that faithful and multiple-mate females differed in age (see *Methods*). This suggests that female paint use is influenced less by overall experience associated with female age than by recent mate searching experience. Information from searches previous to the current year may be less useful to females. For example, when top males that have mated with a particular set of females for multiple years die, those females expand their mate searching (Uy et al., 2001b).

Until recently, avian chemical communication was not well studied, even though it has been shown to occur in a number of social circumstances (reviewed in Hagelin & Jones, 2007), including mating situations (Bonadonna & Nevitt, 2004; Jones, Hagelin, Major, & Rasmussen, 2004). Previous studies have shown that male satin bowerbirds spend 24% of their time at the bower painting (Bravery et al., 2006), that washing paint off of bowers greatly reduces female visits to bowers (Hicks et al., 2013) and that male time spent painting is correlated with male mating success (Robson et al., 2005), suggesting that the information gained from sampling paint is important in mate choice. Although we do not know what females are tasting in paint that might affect their mating decisions, an intriguing possibility is that they are assessing chemical products that reflect the allelic composition of the major histocompatibility complex (MHC) (Bravery et al., 2006; Hicks et al., 2013; Reynolds et al., 2009; Robson et al., 2005). Mate choice related to MHC allelic composition is now well documented (Milinski, 2006) and is often mediated through chemical cues (Wedekind, Seebeck, Bettens, & Paepke, 1995).

Although our study does not directly address the potential chemical function of paint, we were able to study how females use it during mate searching. Our study demonstrates that females that appear more undecided about their choice of mates are more likely to attend to this cue when they visit bowers. We can use this tasting behaviour to predict some female characteristics during mate searching, for example, the number of males that a female mates with or her likelihood of switching among males during her mate search. As more studies are conducted on female behaviours during courtship in other species, it is likely that more active female sampling behaviours will be quantified and examined. We expect that male chemical signals used as display elements will be well-suited for studying active female sampling because of

quantifiable behaviours such as tasting, sniffing or touching associated with sampling these traits. These behaviours have the potential to reveal a great deal about the function of particular male display elements and about female mental states with regard to mate assessment and mate choice.

## Acknowledgments

This work was supported by the National Science Foundation under grant no. 0518844 to G.B. New South Wales National Parks and the Kennedy, Bell and Mulcahy families allowed access to their property and provided other forms of support along with Bill Buttemer and the Veneris family. Metal identification bands were provided by the Australian Bird and Bat Banding Scheme (ABBBS). Jean-François Savard helped supervise the field crew in 2003–2004 and Carrie Long helped supervise in 2004. L. Taylor, M. Terzi, J. Garten, J. Rehm-Lorber, C. Murphy, K. Elliott, G. Gareau, D. Oben-shain, L. Parker, L. Plenderleith, M. South and J. Spence assisted in the field. Thanks also to the University of Maryland undergraduates who watched and coded videotapes. Brian Coyle, Reimi Hicks, Sheila Reynolds, Jean-François Savard and Paul Zwiwers gave valuable comments and support. Special thanks to Julie Hagelin and an anonymous referee for their constructive comments that improved this manuscript.

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