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

ABSTRACT

It can be difficult to assess to what degree a female attends to an individual element of a multicomponent 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 on the degree to which individual females utilize that element while assessing males and how she compares with other females in sampling that element. 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.

25 **KEY WORDS:** Uncertainty, mate assessment, mate choice, mate sampling, mate
26 search, multiple mating, *Ptilonorhynchus violaceus*, satin bowerbird, active sampling,
27 sexual selection
28

Mate searching and assessment are often complicated processes in species where males have multielement 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 & Uetz (1999) found that females of different spider species responded differentially to the individual components of male multimodal display. Coleman, Patricelli, & Borgia (2004) showed that older females used different male display elements than younger females. Yorzinski, Patricelli, Babcock, Pearson & Platt (2013) showed that during courtship female peafowl directed 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. Moreover, we could correlate active female sampling of a display element with particular behaviours that may be influenced by her sampling. 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 male 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 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 foetal development (Singer, Beauchamp, & Yamazaki, 1997). *Drosophila* females touch males to sample cuticular pheromones (Howard, Jackson, Banse, & Blows, 2003) as they do in crickets (Thomas & Simmons, 2009), beetles (Peterson, et al., 2007) and 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, Jones, & Rasmussen, 2003, Hagelin 2006). They are predictive of reproductive success in a common passerine, Dark-eyed Juncos, *Junco hyemalis* (Whittaker, Gerlach, Soini, Novotny, & Ketterson, 2013). In addition, they are suspected in the "exploded lek"

75 system of the endangered Kakapo, *Strigops habroptilus* (Hagelin 2004, Gsell 2012).
76 However, they have not been associated with other lekking birds apart from female
77 “paint” tasting in satin bowerbirds (e.g., Bravery, Nicholls, & Goldizen, 2006; Hicks,
78 Larned, & Borgia, 2013; Robson, Goldizen, & Green, 2005).

79
80 In addition to active sampling of male display traits, female signals to males in
81 response to male display may also reveal females’ mental state. Signals differ from
82 active display trait sampling because they function to influence the behaviour of the
83 displaying male. For example, female cowbirds produce wing strokes in response to
84 males singing attractive songs. This stimulates males to sing more of these effective
85 songs, triggering female copulatory postures (Gros-Louis, White, King, & West, 2003;
86 King, West, & White, 2003; West & King, 1988). Female wolf spiders have receptive
87 postures that have been used to demonstrate preferences for particular components of
88 male display (Hebets & Uetz 1999). Females also often signal readiness to mate (e.g.,
89 Patricelli, Uy, Walsh, & Borgia, 2002; Patricelli, Uy, & Borgia, 2004; Santangelo, 2005;
90 Demary, Michaelidis, & Lewis, 2006).

91
92 Satin bowerbird males have displays involving multiple elements that include the
93 bower, bower decorations, and coordinated visual and vocal displays, which are
94 assessed by females in multiple visits to bowers (Borgia, 1985; Patricelli, et al., 2002;
95 Robson, et al., 2005; Uy, Patricelli, & Borgia, 2000). Studies of satin bowerbirds have
96 demonstrated the value of quantifying variable behaviours during courtship that reveal a
97 female’s mental state. Males court females standing in the bower with energetic dancing

and vocal displays (Coleman, Patricelli, Coyle, Siani, & Borgia, 2007; Loffredo & Borgia, 1986). Females visiting bowers typically start in a standing position and then after several visits, lower into a crouch, indicating her comfort with the male and his display, and finally the female 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, while young females attend 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 and startling signals have allowed us to consider how female behaviour in the bower can indicate their reaction to male displays and their readiness to mate. Even with this information, however, it can be difficult to determine to what degree and when in the mate selection process females are 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 24% of the total amount of time spent at their bowers, roughly equal to the time spent on bower building. In addition, Robson, et al. (2005) found that male painting rate was correlated with

121 mating success. During visits to bowers, females nip at the bower walls and swallow,
122 appearing to taste the paint (Bravery, et al., 2006; Hicks, et al., 2013; Robson, et al.,
123 2005). Such tasting occurred at 39% of all courtship visits (Bravery, et al., 2006;
124 Robson, et al., 2005), suggesting that paint may be a chemical signal used in mate
125 assessment. More specifically, Reynolds, et al. (2008) speculate a role for paint in
126 kinship recognition. Hicks, et al. (2013) showed that female return visits to bowers after
127 initial visits were reduced by paint removal.

128
129 Studying female bowerbird sampling of paint offers a potentially important and
130 novel window on female assessment of males. While crouching acts as a signal
131 indicating female comfort and a female's readiness to mate (Patricelli, et al., 2002;
132 Patricelli, et al., 2004), tasting could provide information about how a specific display
133 element, paint, may affect female decision-making during mate searching. In addition,
134 tasting behaviour can occur at any point in the mate searching process, including early
135 during mate searching, or even when the male is absent from his bower. By studying
136 this behaviour, we can examine how tasting relates to mate assessment. We can
137 measure: (1) if and when females taste during mate searching, (2) if individual female
138 differences relate to differences in tasting behaviour, and (3) if individual differences in
139 tasting behaviour can provide insight into a female's mental state during her search.

140
141 Female mate searching can be difficult to quantify. However, in the Wallaby
142 Creek bowerbird population we have been able to uniquely band most females and
143 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, & Borgia (2001a, b) 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., 2008). This narrowing down of male visitation suggests that females become more certain about their mate choice as they progress towards mating. Females vary in the extent that this narrowing process occurs. A subset of singly mating females, referred to as “faithful” females, mate with the same mate from the previous year, with less searching than other females that switch mates between years (Uy, et al., 2000; Uy, et al., 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 more to assess potential mates early in

the mate selection process. This led to the hypothesis that females who tasted in a greater proportion of their visits in mate searching 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 tastes during their visits. Second, based on mating patterns, we assigned females to one of three mating classes which 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 who 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, the 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 were 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, NSW, 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 1977-1987 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: “noncourtship” during 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

In order to examine how female mating class influences tasting behaviour, three exclusive categories were defined. Faithful (F) females were those that mated with the same male in 2003 and 2004 ($N = 13$; see Uy, et al., 2001a). Multiple mate (M) females mated with more than one male in 2004 ($N = 14$; range = 2-3 males, mean $\pm$ s.d. = 2.14 ± 0.36 males). As an intermediate mating class, novel mate (N) females mated with one male in 2004 who was not their mate in 2003, either because she did not mate in 2003 or she switched males between 2003 and 2004 ($N = 38$). Females that did not fit into any of these classes ($N = 21$), such as females who were not present in 2003 (i.e., one-year old females ($N = 4$)) or females who 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), M females visited significantly more males than either F ($U_{mw} = 160.0$, $N_M = 14$, $N_F = 13$, $P = 0.00064$) or N females ($U_{mw} = 409.5$, $N_M = 14$, $N_N = 38$, $P = 0.0025$) and tended to appear more times at bowers (M:F $U_{mw} = 128.0$, $N_M = 14$, $N_F = 13$, $P = 0.071$; M:N $U_{mw} = 431.5$, $N_M = 14$, $N_N = 38$, $P = 0.00062$). 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 F and M females ($U_{mw} = 85.0$, $N_F = 13$, $N_M = 14$, $P = 0.77$). N females were significantly younger than F and M females (N:F $U_{mw} = 151.5$, $N_N = 38$, $N_F = 13$, $P = 0.038$; N:M $U_{mw} = 174.5$, $N_N = 38$, $N_M = 14$, $P = 0.057$).

Statistical analyses

Data were analysed using R v3.2.0 (R Core Team 2015). In order 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 could be greatly affected by the total number of males 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.

Data were checked for normality and homogeneity of variances, and these parametric test assumptions were violated. Therefore, the data were rank transformed and the appropriate nonparametric tests were used. The Mann-Whitney U test (U_{mw}) was used to determine differences between independent groups, the Wilcoxon signed rank test (W) was used to determine differences between paired observations, and the Spearman's rank correlation (r_s) was used to test for correlations.

We predicted *a priori* that the proportion of visits with tastes would be positively related to behaviours that suggest 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 (Figure 1). We found that tasting occurred proportionately less during visits ending in copulations compared to either visits without courtships ($W=398.0$, $N=86$, $P=0.0048$) or visits with courtships ($W=513.5$, $N=86$, $P=0.0081$). There was no significant difference between the proportion of visits with tastes where courtship occurred but did not end in copulation compared to visits without courtships ($W=1010.0$, $N=86$, $P=0.81$).

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 ($r_s = 0.18$, $N = 86$, $P = 0.10$) 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 ($U_{mw} = 713.5$, $N_{nontasting} = 18$, $N_{tasting} = 68$, $P = 0.28$). Nontasting females visited significantly fewer males ($U_{mw} = 317.0$, $N_{nontasting} = 18$, $N_{tasting} = 68$, $P = 0.0013$) and appeared fewer times at bowers ($U_{mw} = 163.5$, $N_{nontasting} = 18$, $N_{tasting} = 68$, $P < 0.0001$) than those females who 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 bower visitation during mate searching.

We next examined how tasting relates to mating class, excluding those females that were not present in 2003 or did not mate in 2004 from the analyses (see Methods). Tasting females were evenly distributed among the three female classes: 85% of faithful (F) females (11/13), 82% of novel mate (N) females (31/38) and 100% of multiple mate (M) females (14/14) tasted at least once during their mate search ($\chi^2_2 = 2.94$, $P = 0.23$). F females did not differ from M or N females in the proportion of males they tasted (F:M $U_{mw} = 86.0$, $N_F = 13$, $N_M = 14$, $P = 0.80$; F:N $U_{mw} = 300.0$, $N_F = 13$, $N_N = 38$, $P = 0.24$), although N females tended to taste a lower proportion of males than M females (N:M $U_{mw} = 175.0$, $N_N = 38$, $N_M = 14$, $P = 0.055$). Nor did F, N and M females differ in the probability of tasting at least once the partner of the male they eventually copulated with

(F:M $U_{mw} = 90.0$, $N_F = 13$, $N_M = 14$, $P = 0.96$; N:M $U_{mw} = 210.0$, $N_N = 38$, $N_M = 14$, $P = 0.19$; F:N $U_{mw} = 281.5$, $N_F = 13$, $N_N = 38$, $P = 0.38$).

We examined how the overall proportion of visits with tastes related to different females' mating classes (Figure 2a). We found no significant difference between the proportion of visits with tastes between F and N females ($U_{mw} = 194.0$, $N_F = 13$, $N_N = 38$, $P = 0.25$). However, they both had a significantly smaller proportion of visits with tastes than M females (F:M $U_{mw} = 19.5$, $N_F = 13$, $N_M = 14$, $P = 0.00050$; N:M $U_{mw} = 124.0$, $N_N = 38$, $N_M = 14$, $P = 0.0033$). Overall, multiple mating females tasted 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 (Figure 2b). There was only one significant difference between visit types within a female mating class: F females had a significantly smaller proportion of courtships ending with copulations with tastes than noncourtship visits with tastes ($W = 1.5$, $N = 13$, $P = 0.013$). There were, however, significant differences among the female mating classes. While F and N females did not differ significantly from each other in the proportion of tasting across any visit type (noncourtship: $U_{mw} = 288.5$, $N_F = 13$, $N_N = 38$, $P = 0.32$; courtship: $U_{mw} = 201.0$, $N_F = 13$, $N_N = 38$, $P = 0.30$; copulation: $U_{mw} = 211.5$, $N_F = 13$, $N_N = 38$, $P = 0.34$), they both differed from M females during courtships and courtships ending with copulation. M females had a greater proportion of courtships and courtships ending in copulations with tastes compared to F (courtship: $U_{mw} = 162.5$, $N_M = 14$, $N_F = 13$, $P = 0.00045$;

copulation: $U_{mw} = 158.0$, $N_M = 14$, $N_F = 13$, $P = 0.00065$) or N (courtship: $U_{mw} = 423.5$, $N_M = 14$, $N_N = 38$, $P = 0.00099$; copulation: $U_{mw} = 381.0$, $N_M = 14$, $N_N = 38$, $P = 0.0096$) females.

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 ($r_s = 0.30$, $N = 86$, $P = 0.0055$) 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, M females had more excess switches than either F or N females (M:F $U_{mw} = 170.5$, $N_M = 14$, $N_F = 13$, $P < 0.0001$; M:N $U_{mw} = 453.5$, $N_M = 14$, $N_N = 38$, $P < 0.0001$). F and N females did not differ in their number of excess switches (F:N $U_{mw} = 187.0$, $N_F = 13$, $N_N = 38$, $P = 0.14$). Similar results were found when considering the number of switches per male visited, although in this case, all mating classes differed (Figure 3; M:F $U_{mw} = 170.5$, $N_M = 14$, $N_F = 13$, $P < 0.0001$; M:N $U_{mw} = 455.5$, $N_M = 14$, $N_N = 38$, $P < 0.0001$; F:N $U_{mw} = 156.5$, $N_F = 13$, $N_N = 38$, $P = 0.044$).

We next examined the relationship between mating class and the proportion of males rejected (F: 0.25 ± 0.36 , N: 0.47 ± 0.31 , M: 0.42 ± 0.25 (mean $\pm$ s.d.)). F females had a tendency to reject a lower proportion of males than N females ($U_{mw} = 161.5$, $N_F = 13$, $N_N = 38$, $P = 0.054$), but this probably reflects them visiting fewer (and often only one) males ($U_{mw} = 161.0$, $N_F = 13$, $N_N = 38$, $P = 0.054$). There were no significant differences between the other mating classes in the proportion of males they rejected (F:M $U_{mw} = 62.5$, $N_F = 13$, $N_M = 14$, $P = 0.15$; N:M $U_{mw} = 307.5$, $N_N = 38$, $N_M = 14$, $P = 0.38$).

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 females 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 N 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 who 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 in 2003, this difference was not statistically significant ($W = 142.0$, $N = 30$, $P = 0.39$). However, perhaps many females still taste bowers they have sampled the previous year to update their information or confirm it is still the same male. A different test of the familiarity hypothesis is that females should taste less as they become more familiar with the male. One prediction of this hypothesis is that females should taste more their first visit with a male than the second, 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 ($r_s = -0.15$, $N = 86$, $P = 0.16$). There was a tendency for 20+ year-old females to taste less than other females ($U_{mw} = 44.5$, $N_{old} = 3$, $N_{younger} = 83$, $P = 0.058$), 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 were able to show that females with more information about high quality mates 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 mates 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 uncertainty for several reasons. First, male satin bowerbird display sites 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 view courtship by several males. Second, because we could continuously monitor behaviour at bowers with video cameras, we could quantify female active sampling of paint on the bower wall as well as reconstruct patterns of female visitation at bowers to construct our different measures of uncertainty. Lastly, because paint is applied to bower walls before courtship begins it is accessible to females throughout courtship so that she is in full control of when sampling occurs.

393

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

410

411 Other alternative hypotheses are less well supported. For example, some
412 females may be less adept at assessing chemical signals leading them to taste more.
413 This would either predict a negative relationship between tasting and age, or if female
414 inability to assess chemical signals cannot be improved over time, consistency in tasting
415 behaviour between years. We do not have evidence for age-related change in tasting

(see also below). While we do not have multiple years of data on tasting available, 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 who search and taste less commonly mate with high success males (Uy et al. 2000) and it is unlikely that less motivated females would be better at finding high success 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 or courtships that did not end in copulations. Tasting was only reduced during courtships that ended in copulations. During these visits, females have already completed the construction of their nests, 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 her 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 and startling are signals of female comfort with male display or readiness to mate (e.g., Patricelli, et al., 2002; Patricelli et al., 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 the finding that females taste more in visits that did not end in copulations compared to those visits where females

made a final mating decision. Even stronger support of this hypothesis is that females taste less their second visit to a particular male than on their first visit. These data support the claim that male display traits are more actively sampled by information-limited females and sampling is reduced as uncertainty decreases. However, females also may differ in their ability to reduce uncertainty. This may occur for several reasons, including: (1) females may vary in their ability to remember male trait values from previous visits, or (2) low information females forced to search among more males may lose track of their previous visits thus lowering their ability to resolve uncertainty in mate choice. 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 ability to assess males and 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 in female tasting behaviour. In addition, we did not find that F and M 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. For example, when top males who 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 (review: Hagelin & Jones 2007), including mating situations (Bonadonna & Nevitt 2004; Jones, Hagelin, Major, & Rasmusen, 2004). Female sampling behaviour of bower paint during visits to male bowers suggests that paint is a chemical signal important in mate assessment. In addition, the observations that males spend about a quarter of their time at the bower painting (Bravery, et al., 2006), that washing paint off of bowers greatly affects female visitation at bowers (Hicks, et al., 2013), and that male time spent painting is correlated with male mating success (Robeson, et al., 2005), suggests 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., 2008; 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 are able to study how females use it in relation to other display elements. Our study suggests that females use paint during mate assessment and that females who appear more undecided about their choice of mates are more likely to attend to this cue when they visit bowers. We can use this behaviour to predict some female characteristics during mate searching, for example, the number of males she mates with

485 or her likelihood to switch more among males during mate searching. As more studies
486 are conducted on female behaviours during courtship in other species, it is likely that
487 more active female sampling behaviours will be quantified and examined. We expect
488 that male chemical signals used as display elements in courtship will be well-suited for
489 studying active female sampling because of quantifiable behaviours such as tasting,
490 sniffing, or touching associated with sampling these traits. These behaviours have the
491 potential to reveal a great deal about the function of particular male display elements
492 and about female mental states with regard to mate assessment and choice.
493

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586

587 **Figure 1.**

588
589 **Figure 1.** Mean proportion of each visit type in which tasting occurred, subdivided by
590 visit type. Error bars are 95% confidence intervals.

592 **Figure 2a.**

593
594 **Figure 2. (a)** Mean proportion of visits in which tasting occurred, subdivided by female
595 mating class. Error bars are 95% confidence intervals.

596 **Figure 2b.**

597
598 **Figure 2. (b)** Mean proportion of visits in which taster occurred, subdivided by female
599 mating class and visit type. Error bars are 95% confidence intervals.

601 **Figure 3**

602
603 **Figure 3.** Mean excess switches, subdivided by female mating class. Error bars are
604 95% confidence intervals.