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RESEARCH ARTICLE

Spontaneous choices for insect-pollinated flower shapes by wild non-eusocial halictid bees

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ABSTRACT

The majority of angiosperms require animal pollination for reproduction, and insects are the dominant group of animal pollinators. Bees are considered one of the most important and abundant insect pollinators. Research into bee behaviour and foraging decisions has typically centred on managed eusocial bee species, including *Apis mellifera* and *Bombus terrestris*. Non-eusocial bees are understudied with respect to foraging strategies and decision making, such as flower preferences. Understanding whether there are fundamental foraging strategies and preferences that are features of insect groups can provide key insights into the evolution of flower–pollinator co-evolution. In the current study, *Lasioglossum* (*Chilalictus*) *lanarium* and *Lasioglossum* (*Parasphecodes*) sp., two native Australian generalist halictid bees, were tested for flower shape preferences between native insect-pollinated and bird-pollinated flowers. Each bee was presented with achromatic images of either insect-pollinated or bird-pollinated flowers in a circular arena. Both native bee species demonstrated a significant preference for images of insect-pollinated flowers. These preferences are similar to those found in *A. mellifera*, suggesting that flower shape preference may be a deep-rooted evolutionary occurrence within bees. With growing interest in the sensory capabilities of non-eusocial bees as alternative pollinators, the current study also provides a valuable framework for further behavioural testing of such species.

KEY WORDS: Angiosperms, Behaviour, Decision making, Foraging, Pollinators, Native bees

INTRODUCTION

Animal visitations are important for the successful pollination of many angiosperms. About 300,000 animal species (Kearns et al., 1998) are involved in pollinating approximately 87% of angiosperms (Ollerton et al., 2011). Bees are considered one of the most important insect pollinators of angiosperms (Ballantyne et al., 2017); however native wild bees are often overlooked in research, pollination and crop production, and in economic value (Batley and Hogendoorn, 2009; Houston, 2018). This is despite research

suggesting that wild bees and insects may be more effective at agricultural pollination than managed honeybees (*Apis mellifera*) in some environments depending on the crop, such as in Macadamia crops (Heard, 1994; Karasiński, 2018), and tomato crops, which honeybees cannot pollinate because of an inability to perform buzz pollination (Bell et al., 2006; Hogendoorn et al., 2006). The focus on eusocial bee research has resulted in most pollinator models (Bukovac et al., 2013; Dorin et al., 2018; Pasquaretta et al., 2017) and bee-tracking software (Marchal et al., 2020; Pasquaretta et al., 2017; Ratnayake et al., 2020) being built on the behaviour and foraging knowledge of *A. mellifera* or *Bombus terrestris*. Therefore, considering the current and potential future value of native non-eusocial bees (Houston, 2018; Michener, 2000), more research into their foraging behaviour and floral preferences is an important topic. Eusocial bees live cooperatively in a group, usually with one reproductively active female and several reproductive males with a division of labour among the non-reproductive workers (e.g. honeybees and bumblebees). Non-eusocial bees encompass all bees which are not eusocial and can include a range of sociality levels (subsocial, semisocial, quasisocial) as well as solitary bees.

Studies examining important behaviour such as foraging, floral preferences, nesting and pollination in non-eusocial bees using controlled parameters are particularly scarce (De Araujo et al., 2020; Loukola et al., 2020). Such behaviour has been well studied in eusocial species such as *A. mellifera* (for examples, see Garcia et al., 2019; Giurfa et al., 1995; Howard et al., 2019d; Lehrer et al., 1995; Martin, 2004) and *B. terrestris* (for examples, see Essenberg et al., 2015; Lihoreau et al., 2016; Lunau, 1991; Pasquaretta et al., 2019; Rodríguez et al., 2004; Spaethe et al., 2001; Whitney et al., 2008). Although there are studies reporting the foraging and pollination behaviours of non-eusocial bees in field conditions (Stone et al., 1999; Welsford and Johnson, 2012; White et al., 2001) and foraging arenas (Dukas and Real, 1991), more controlled psychophysics experiments (Howard, 2021; Howard et al., 2021a; Menzel et al., 1988; Welsford and Johnson, 2012) are currently lacking.

Many animal pollinators exhibit preferences for floral characteristics such as pattern, size and shape (Dafni and Kevan, 1997; Johnson and Dafni, 1998; Lehrer et al., 1995). Bees utilize flower signals, cues and traits in order to make decisions on which flowers to visit, including scent (Raguso, 2008), colour (Giurfa et al., 1995), shape (Howard et al., 2019d; Lehrer et al., 1995), size (Martin, 2004), quantity of flowers (Howard et al., 2020) and/or symmetry (Giurfa et al., 1996). Honeybees show preferences for flower shape characteristics including flower-like shapes with circularly organized elements which radiate outwards (Lehrer et al., 1995), radial symmetry (Giurfa et al., 1996; Lehrer, 1999) and larger flower sizes (Martin, 2004). Flower shape/morphology is a potentially important signal and/or cue to pollinators as flower morphology can constrain access to some morphologically complex flower species (Krishna and Keasar, 2018), leading to pollinators not receiving

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nectar or pollen rewards despite the output of energy while attempting to forage on an unsuitable flower. In a recent psychophysics experiment, honeybees demonstrated a preference for non-rewarding images of insect-pollinated flowers over those of bird-pollinated flowers, despite having no familiarity with the specific species of flowers tested (Howard et al., 2019d), indicating that specific shape cues may be used to find suitable flowers. It is not known whether these preferences are particular to honeybees alone, perhaps reflecting cognitive capacities related to their eusociality, or whether they are fundamental preferences associated with the foraging behaviour of bees, or insect pollinators in general. Such a finding would suggest a much longer evolutionary association between flower form and insect pollinator preferences, which could provide a key insight into the co-evolutionary history of flowering plants and their insect pollinators (van der Kooi and Ollerton, 2020).

Australia is home to approximately 2000 native bee species (Houston, 2018); however, for many species, we lack information is lacking on the biology, distribution, breeding behaviour, habitat requirements and crop pollination abilities (Batley and Hogendoorn, 2009; Houston, 2018). Nevertheless, there is growing interest in understanding these topics in native bees (for examples, see Bull and Schwarz, 1997; Cunningham et al., 2013; Dorey, 2021; Halcroft et al., 2011, 2013; Heard and Dollin, 2000; Hurst et al., 1997; Indsto et al., 2006; Joyce and Schwarz, 2006, 2007; Kayaalp and Schwarz, 2007; Langer et al., 2006; Leys, 2000; Neville et al., 1998; Rehan et al., 2010; Schwarz and O'keefe, 1991; Schwarz et al., 1996; Smith and Mayfield, 2018; Tierney et al., 1997; Walker, 1993). The current lack of information across the many diverse bee species is thought to be a result of a dearth of taxonomic expertise and funding (https://www.abc.net.au/news/2020-09-10/bee-taxonomy-in-australia-a-dying-art/12647676; Sands, 2018; Saunders et al., 2021; Taylor et al., 2018). In Australia, the native eusocial stingless bee *Tetragonula carbonaria* (previously *Trigona carbonaria*) has been studied for orientation and flight range (Leonhardt et al., 2016; Smith et al., 2017), colour preferences (Dyer et al., 2019, 2016a), flower constancy behaviour (White et al., 2001), nectar temperature preference (Norgate et al., 2010), pollination effectiveness (Heard, 1994) and flower detection abilities (Dyer et al., 2016b), but studies on other native species, particularly non-eusocial species, are lacking, although recent work has examined colour learning and preferences in native non-eusocial halictid bees (Howard, 2021; Howard et al., 2021a). Other studies on native Australian bee foraging behaviour have mainly centred on their involvement in or potential to contribute to crop pollination. Past work has examined the benefit of *Xylocopa* and *Amegilla* in the pollination of tomatoes (Bell et al., 2006; Hogendoorn et al., 2010, 2007, 2006, 2000) and other native bees for the pollination of other crops (Heard, 1999), such as lucerne (alfalfa) (Bray, 1973; Hogendoorn, 2018; Hogendoorn and Keller, 2012). There are approximately 11 species of native eusocial bee in Australia, with the others being solitary or of varied levels of sociality (Houston, 2018). The paucity of information on native Australian bees is considered one of their biggest threats (Batley and Hogendoorn, 2009; Sands, 2018; Taylor et al., 2018). The current study aimed to examine floral shape preference in the native non-eusocial bees *Lasioglossum* (*Chilalictus*) *lanarium* (Smith 1853) and *Lasioglossum* (*Parasphecodes*) sp. Smith 1853 (family: Halictidae), thereby expanding our knowledge of native plant–pollinator systems and broadening our understanding of floral preferences across bee species of different social structures.

Halictids are short-tongued bees and the majority nest in the ground, with a few burrowing into rotten branches, logs or stumps (Houston, 2018). *Lasioglossum* predominantly nest communally, with varying degrees of sociality (Schwarz et al., 2007). The genus is found across Australia (Danforth and Ji, 2001). *Lasioglossum lanarium* is a ground-nesting, generalist bee species, foraging on multiple flowering plant species, and is widespread across Australia (Atlas of Living Australia, accessed 16 February 2021: https://bie.ala.org.au/species/urn:lsid:biodiversity.org.au:afd.taxon:8634ccfb-2dea-48d4-8853-6f91018440f2). This species shows evidence of some sociality (Houston, 2018) by exhibiting communal nesting with aggregations of females but it does not nest in large hives with a division of labour system as in eusocial bee species. *Lasioglossum* (*Parasphecodes*) sp. is also a ground-nesting bee and identification to species level is not possible at this time as the subgenus is undergoing a taxonomic revision (Ken Walker, Museums Victoria, personal communication); however, the species was observed foraging on multiple plant species (S.R.H., personal observation), and has no morphological adaptations to suggest specialization.

In the current study, we tested whether two species of non-eusocial, ground-nesting bees, *L. lanarium* and *L.* (*Parasphecodes*) sp., demonstrated preferences for specific shapes of native Australian flowers, which were either exclusively insect or bird pollinated. Flowers were presented to bees as achromatic images, which removed all potential cues of scent and colour, to examine the overall shape/morphology preferences of native bees for native flowers in Australia. Using images of flowers to examine floral preferences and recognition is an appropriate method as bumblebees view images and real flowers as similar (Thompson and Plowright, 2014) and honeybees previously showed significant preferences for different flower images, as discussed above (Howard et al., 2019d). Although some insect- and bird-pollinated flowers can share similar morphologies, flowers pollinated by different pollinator groups often have different morphologies (Cronk and Ojeda, 2008), which may influence pollinator choice and flower recognition. As past research has demonstrated that honeybees have preferences for certain flower shapes and patterns (Dafni and Kevan, 1997; Giurfa et al., 1996; Howard et al., 2019d; Johnson and Dafni, 1998; Lehrer, 1999; Lehrer et al., 1995), wild non-eusocial bees should demonstrate a similar preference in theory and *L. lanarium* has previously shown a preference for coloured card stimuli representing flower colour signals (Howard et al., 2021a). This study serves to expand our knowledge about foraging decisions and floral preferences in understudied Australian native bees, with the aim of bridging the research gap between foraging behaviour in eusocial and non-eusocial bees. On a global scale, data on bee preferences in Australia serve as an important point of comparison for international studies on how and why flower traits have evolved as a result of the long-term geological isolation of the Australian island continent (Dyer et al., 2012, 2016a,b; Shrestha et al., 2013), and potentially phylogenetically conserved processing mechanisms in bee species (Briscoe and Chittka, 2001).

MATERIALS AND METHODS
Study species and collection

Lasioglossum lanarium and *L.* (*Parasphecodes*) sp. are native Australian bees in the family Halictidae, genus *Lasioglossum* and subgenera *Chilalictus* and *Parasphecodes*, respectively.

In the current study, 20 individuals of *L. lanarium* and 20 individuals of *L.* (*Parasphecodes*) sp. were collected in southeast Melbourne, Australia, on the day of experiments during August–September 2020. Experiments for the two species were conducted simultaneously during those months and species were
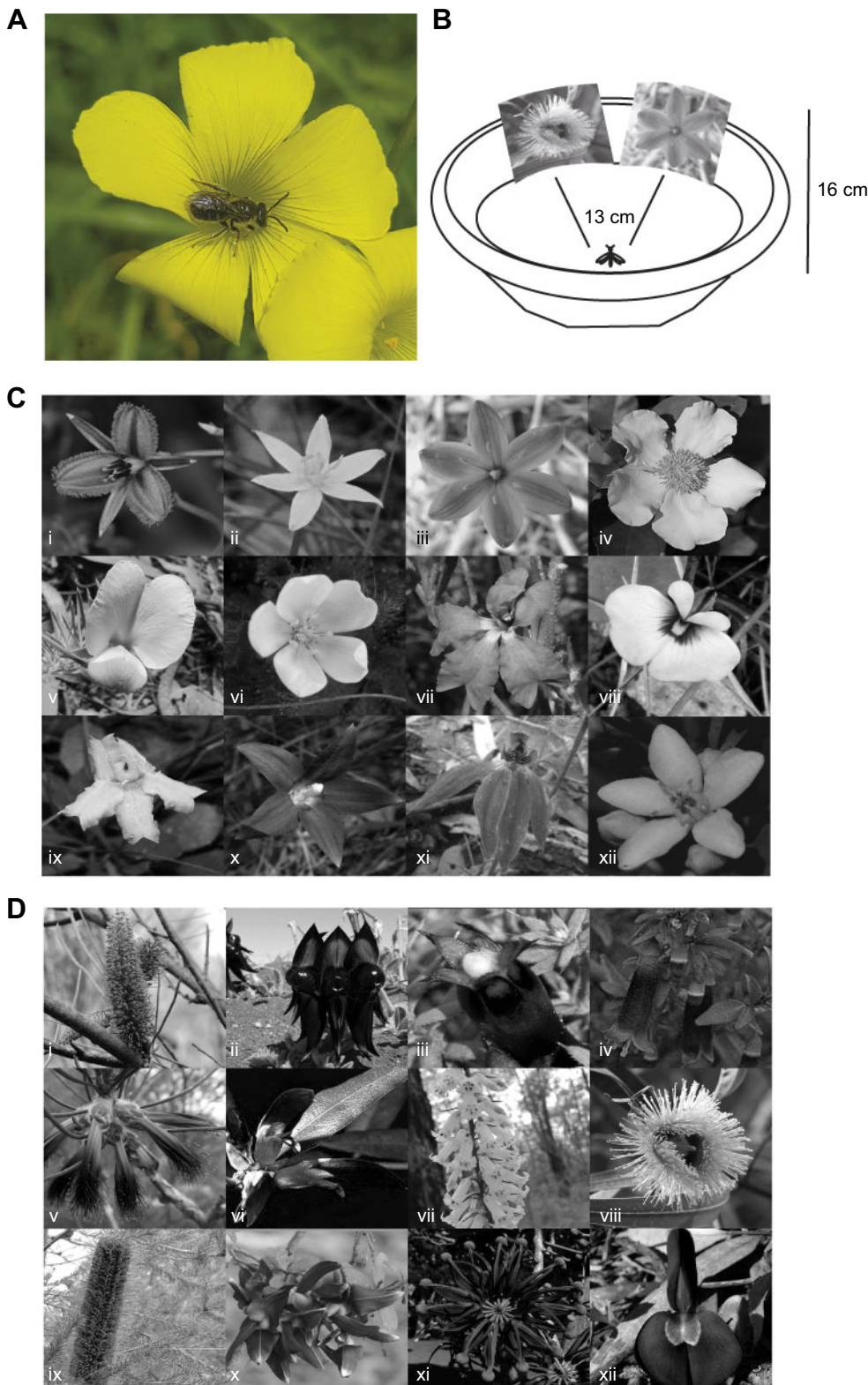
Fig. 1. Species, apparatus and stimuli used in the study. (A) *Lasioglossum lanarium* foraging on *Oxalis pes-caprae*. (B) Preference test for *L. lanarium* and *Lasioglossum* (*Parasphecodes*) sp. between insect- and bird-pollinated achromatic flower images. At the beginning of the trial, the bee is placed at one end of the arena, 13 cm away from the stimuli. A choice is recorded for either flower when the bee climbs onto the stimulus. (C,D) Achromatic images of flowers used in the experiment which were exclusively (C) insect pollinated or (D) bird pollinated. (C) Species names: (i) *Thysanotus juncifolius*, (ii) *Tricoryne elatior*, (iii) *Chamaescilla corymbosa*, (iv) *Hibbertia scandens*, (v) *Gompholobium huegelii*, (vi) *Drosera whittakeri*, (vii) *Dampiera stricta*, (viii) *Eutaxia microphylla*, (ix) *Goodenia lanata*, (x) *Wahlenbergia gloriosa*, (xi) *Caladenia carnea* and (xii) *Philotheca myoporoides*. (D) Species names: (i) *Hakea francissiana*, (ii) *Swainsona formosa*, (iii) *Astroloma ciliatum*, (iv) *Corea pulchella*, (v) *Calothamnus rupestris*, (vi) *Gastrolobium celsianum*, (vii) *Epacris impressa*, (viii) *Eucalyptus* sp., (ix) *Banksia ericifolia*, (x) *Templetonia retusa*, (xi) *Stenocarpus sinuatus* and (xii) *Kennedia prostrata*.

pseudo-randomized for testing sequence on each day of testing. Male and female *L. lanarium* were collected on the introduced weed *Oxalis pes-caprae* (Fig. 1A), while female *L.* (*Parasphecodes*) sp. were collected returning to their nests. Bees were captured in small transparent plastic vials with air holes and transported at ambient temperature (20–30°C) to a testing arena; behavioural assays were conducted within 24 h of capture. Male and females of *L. lanarium* were not separated by sex. Bees were nectar deprived for at least 2 h before the experiment began. All animal care was in accordance with institutional guidelines. Formal ethics approval was not required for invertebrate behavioural testing.

Apparatus

Pilot experiments were conducted with multiple arenas to determine which size and shape would be best for the behavioural testing described in the current experiment and were the same (Howard, 2021) or similar (Howard et al., 2021a) to recent studies on *L. lanarium* and *L.* (*Parasphecodes*) sp. During pilot experiments, individuals responded to preference tests best within a circular arena; thus, this apparatus design was used for testing. The arena was constructed of white plastic and was 16 cm in diameter (Fig. 1B; Howard, 2021). All bees were individually tested. Experiments were conducted throughout the day (11:00–16:00 h) under natural daylight conditions.

Stimuli

Stimuli were achromatic images of native Australian insect- or bird-pollinated flowers collected in past studies (Burd et al., 2014; Shrestha et al., 2013) and used in a previous preference experiment on honeybees (Howard et al., 2019d). There were 24 flower images overall, of which 12 were exclusively insect pollinated and 12 were exclusively bird pollinated (Fig. 1C,D). Each image was cropped to 6×6 cm. All images presented flowers on natural backgrounds (Fig. 1C,D) to ensure the stimuli were as visually similar to the natural environment as possible, in regards to floral backgrounds. The pairs of images (insect-pollinated flower versus bird-pollinated flower) presented were randomized in terms of flower species per individual bee. The pair of stimuli used for each individual bee was the same for all 10 choices of that bee. A previous study showed that there were no significant differences in flower line length (flower perimeter), flower size (area) or image contrast between the insect- and bird-pollinated images; thus, these parameters were not a predictor of preference for bees (Howard et al., 2019a,b,c,d). Specifically, contrast was measured as whole pixel intensity and did not differ between the pictures (Howard et al., 2019a,b,c,d). Thus, this effect was eliminated to bias bee choice. We chose to compare the image contrast for the entire image rather than for each pixel as it is not possible that bee vision can resolve each individual pixel in an image, and thus the contrast or brightness of each pixel. Therefore, the overall stimulus measure was taken, as is best practice for bee studies (see Dyer et al., 2008; Howard et al., 2019a,b,c,d; Zhang et al., 2004). Furthermore, some Hymenopteran (e.g. *A. mellifera* and *Polistes fuscatus*) prefer to process images globally (as a whole) rather than locally (Avarguès-Weber et al., 2015; Tibbetts et al., 2021). Printing and controls of the stimuli were identical to the previous honeybee study (Howard et al., 2019d). Although there were differences between the testing apparatus and the number of stimuli displayed, in both the current study and previous study on honeybees, stimuli were vertically presented to bees and only two different flower stimuli were presented at a time. Differences between the methods included using freely walking *Lasioglossum* bees as opposed to free-flying honeybees, the apparatus used and the mode of testing – whereas honeybees were first attracted to the apparatus with sucrose solution, *Lasioglossum* bees were placed into an arena to record spontaneous choices without any reward.

Preference tests

Each bee was placed 13 cm away from the two stimuli presented (one insect-pollinated flower versus one bird-pollinated flower), resulting in a global visual angle of 26 deg for each of the respective images in a given test. The petal (local/elemental feature) of the smallest insect-pollinated flower (Fig. 1Cii, *Tricoryne elatior*) was about 2 cm and had a visual angle greater than 8 deg, which is above the minimum object detection threshold previously measured for *A. mellifera*, at a visual angle of 5 deg (Avarguès-Weber et al., 2015),

and well above the 1.5 deg acuity threshold for processing patterned visual information (Srinivasan and Lehrer, 1988). In the current study, a coarser minimum visual angle than both colour and pattern stimuli in other bee species was used for *L. lanarium* and *L.* (*Parasphecodes*) sp. as no data are currently available on their acuity, and pilot tests demonstrated that 6×6 cm stimuli at an initial visual angle of 26 deg (and an approximate local feature visual angle of 8.7 deg) were attractive to these species.

Each bee was placed into the arena using a toothpick, which they readily climbed onto for transport in and out of the arena. The trial lasted until the bee made a choice (all bees did so within the range of approximately 5 s to 60 s). Bees from both species appeared attracted to stimuli, showing a motivation to walk towards them upon placement into the arena as seen in Movie 1 and in recent experiments on these two species (Howard, 2021; Howard et al., 2021a). A choice was recorded once a bee climbed onto the flower stimulus (see Movie 1). The choice of a bee for a flower stimulus was recorded when a clear touch of the image was made, which is a common metric for bee behavioural studies (Chittka et al., 2003; Giurfa et al., 2001; Howard et al., 2020; Perry and Barron, 2013; Raine and Chittka, 2008). Bees climbed onto the stimulus and either climbed over it or stopped on the image (as seen in Movie 1). After a choice was made, the bee was collected from the stimulus, the apparatus and stimuli were cleaned with 70% ethanol and dried, stimuli were randomized for side placement, and then the bee was placed back into the starting position for the next trial (Fig. 1B). A total of 10 choices were recorded per bee as is common in unreinforced tests with *A. mellifera* (Howard et al., 2019a,b,c, 2020), *L. lanarium* (Howard, 2021; Howard et al., 2021a) and *L.* (*Parasphecodes*) sp. (Howard et al., 2021a), as well as other animals (Prendergast et al., 2016).

General description of working with non-eusocial bees

Given the rarity of behavioural data available from non-eusocial bee species, we would like to briefly describe our experiences to help enable future work. First, it was possible to capture *L. lanarium* by slowly approaching flowers they were on and collecting the flower and bee together in a vial. For *L.* (*Parasphecodes*) sp., we had to collect bees as they landed near their nest, but before they entered the nesting cavity. Bees were transported in vials in a dark bag with zero mortality during or after transport. On placement into the arena, bees opted to walk towards the stimuli, seemingly a visually driven response to the stimuli. In cases where bees tried to fly or exhibited escape behaviour, which was rare, a clear UV-transparent plastic lid was placed on the arena. Once bees had clearly made a choice, they were collected onto the toothpick while stimuli were cleaned and re-arranged in a pseudorandom position. The bee was then placed back onto the starting area for the next trial.

Statistical analysis

To determine whether bees preferred insect- or bird-pollinated images, we analysed the test data by employing a generalized linear mixed-effects model (GLMM) with a binomial distribution including only the intercept term as fixed factor and individual bee ID (subject) as a random term to account for repeated measures. The proportion of choices for the insect-pollinated flower (mean proportion of correct choices, MPCC) recorded from the tests was used as the response variable in the model. The Wald statistic (z) tested whether the mean proportion of insect-flower choices recorded from the preference test, represented by the coefficient of the intercept term, was significantly different from chance expectation, i.e. H_0: MPCC=0.5.

To assess whether there was a difference in flower choice between bee species, we employed a GLMM including bee species as fixed factor and subject as a random term to account for repeated measures. As above, the proportion of choices for the insect-pollinated flower was the response variable.

Both models were estimated using the routine 'glmer' available as part of the 'lme4' package written for the R statistical language, run in R version 4.0.3 (Bates et al., 2015; http://www.R-project.org/).

Model diagnostics were assessed using the package 'DHARMa' (http://florianhartig.github.io/DHARMa/) using simulation-based tests of the residuals to test for over/underdispersion. Models for both species did not exhibit significant dispersion (both ratio obs: sim=1.004; P=1).

RESULTS

Lassioglossum lanarium showed a significant 62.5% preference for insect-pollinated flowers (z=3.497, P<0.001, n=20, confidence interval, CI: 0.557–0.690; Fig. 2) over bird-pollinated flowers. *Lassioglossum* (*Parasphecodes*) sp. also showed a 62.5% preference for insect-pollinated flowers (z=3.497, P<0.001, n=20, CI: 0.555–0.696; Fig. 2). The two bee species therefore showed the same degree of preference. Data from both species were overdispersed (1.33 for both species), but within a reasonable range to perform the analysis.

DISCUSSION

Lasioglossum lanarium and *L.* (*Parasphecodes*) sp. significantly preferred the achromatic images of native Australian insect-pollinated flowers to bird-pollinated flowers. Image controls have determined that the preference could not be due to brightness or contrast, line length of the flowers (perimeter), or flower size (Howard et al., 2019d). The use of well-specified achromatic images also enables the total exclusion of potential olfactory cues present in natural environments. Additionally, a previous study demonstrated that another bee species, *A. mellifera*, had no preference for brightness, elongation or number of flowers (Howard et al., 2019d) and available evidence suggests bees are poor at processing

brightness cues as useful information when finding flowers (Ng et al., 2018; van der Kooi et al., 2019), nor do bees process brightness differences even when intensity is reversed (Dyer et al., 2007, 2010; Giger and Srinivasan, 1995). Therefore, similar visual preferences for the same flower morphologies arise in both eusocial and non-eusocial bees, which lack the communication and co-operation observed in eusocial hives (e.g. waggle dance: Biesmeijer and Seeley, 2005; Riley et al., 2005; or scent marking by conspecifics: Free and Williams, 1983; Giurfa and Núñez, 1992; Giurfa et al., 1994; Stout and Goulson, 2001).

The preference of both *L. lanarium* and *L.* (*Parasphecodes*) sp. for native Australian insect-pollinated flower images could be a result of either familiarity with foraging on related flowers or an innate preference for certain flower shapes, similar to innate preferences observed for flower colour in both Australian native stingless (*T. carbonaria*) bees and European honeybees (Dyer et al., 2016a; Giurfa et al., 1995). If the latter case is true, then this suggests that recognition and preference for certain flower shapes could be an adaptive behaviour that was present early in the evolutionary history of bees (Dafni et al., 1997; Lehrer, 1999), and possibly much earlier. As suggested in Howard et al. (2019d), there are two potential evolutionary mechanisms. The first hypothesis is that flowers evolved to exploit bee floral preferences, such as shape (Gegear et al., 2017; Lehrer et al., 1995). This scenario is plausible as angiosperms are predicted to have arisen during the Late Triassic period, while Hymenoptera arose millions of years prior during the Permian time period (van der Kooi and Ollerton, 2020). The second hypothesis is that bees have evolved to prefer flower shapes of insect-pollinated flowers as the nutrition is generally more accessible to them than in bird-pollinated flowers because of the morphology of the flower (Brown et al., 2011). The combination of these two hypotheses – co-evolution of angiosperms and bee pollinators (Johnson and Anderson, 2010) – is also a possible explanation for the preference observed for insect-pollinated flower shapes in both non-eusocial and eusocial bees. The co-evolution of specific plant and pollinator morphologies is observed in multiple cases; for example, pollinator tongue length and flower tube length (Anderson and Johnson, 2008). The evidence that two species of native Australian bees (having evolved with the flowers in Australia) and the European honeybee (tested in Germany with no opportunity to encounter these flower species) have similar preferences for flower shapes of native Australian plants, despite 120 million years of evolution since their last common ancestor (Cardinal et al., 2010), suggests that the preference could be a deep-rooted evolutionary occurrence, although testing more evolutionarily separate bee species, particularly from more ancient lineages, such as Melittinae and Dasypodainae (both Melittidae) (Husemann et al., 2021), would be required to confirm this hypothesis. It should be noted that besides innate shape and colour preferences, it is likely that insect visitors are first attracted to the scent that certain flowers produce, which is potentially important in the current study. For example, many native Australian bees specialize, and forage in high abundance, on species with generalized, or even 'bird syndrome' shapes (e.g. some *Eucalyptus*, some *Eremophila*, *Calothamnus*, *Callistemon*, *Banksia*, *Grevillea*) (e.g. Hingston et al., 2004; Horskins and Turner, 1999; Prendergast, 2020; K.P., personal observation of native Australian bees visiting *Eucalyptus* plant species) although this may be due to olfactory cues rather than flower shape preferences. Indeed, recent research shows that both visual and olfactory cues (colour and scent) are very important in attracting pollinators in some environments (Kantsa et al., 2017, 2018) and so
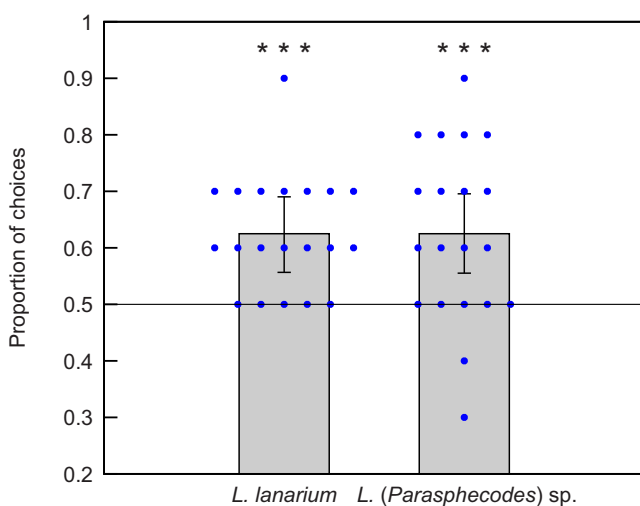

Fig. 2. Mean proportion of choices (±95% confidence interval) for the insect-pollinated flower images by *L. lanarium* and *L.* (*Parasphecodes*) sp. during the preference test. Horizontal black line shows chance expectation at 0.5. Blue circles indicate the raw data from each individual bee (*n*=20 per species) as a bee-swarm plot. Significant difference from chance level performance is indicated by ***P≥0.001.

future work on how non-eusocial bees may process scent will be of value. To date, this has proven to be challenging in lab conditions as volatile scent is diffuse and hard to control with free-moving insects, whilst in vision studies, bees are very difficult to test while harnessed (Avarguès-Weber and Mota, 2016).

Attraction to floral shape conceivably acts at a later stage in the chain of behavioural decisions that an actively foraging bee makes, becoming pertinent once it approaches a flower based on more general long-distance cues such as scent and colour (Chittka and Raine, 2006; Dyer and Chittka, 2004). Such preferences can help a bee find a flower amongst the large amount of noisy information in the environment, and if such a flower is rewarding, this would allow subsequent learning to enable efficient foraging of honest signalling flowers. We standardized the start distance to 13 cm (Howard, 2021), but further studies would be useful to determine at what distances different bee species can resolve images, and at what stage in the foraging choice sequence shape becomes a pertinent cue (Dafni et al., 1997; Nityananda et al., 2014). Moreover, whilst we isolated shape, these cues do not act alone, and shape may serve to reduce uncertainty of other cues (Leonard et al., 2011).

A current challenge in working with solitary or non-eusocial bees is the requirement to source insects from the wild, as opposed to eusocial bees that can be reared in conditions that totally exclude the experience of visiting real flowers. This means it is difficult to exclude influences of associative learning that might influence preferences. We were also not able to determine levels of starvation/satiation or energetic stress, and it is conceivable that these factors influence a bee's foraging choices, as has been found in *A. mellifera* (Katz and Naug, 2015; Mayack and Naug, 2015). Furthermore, as males and females of *L. lanarium* were not separated, we could not account for different preferences based on sex and it is possible that sex had an impact on flower choice. For example, males of *Andrena* (*Hesperandrena*) *limnanthis* prefer white traps over blue and yellow traps, while females strongly prefer white and blue traps, but not yellow traps (Leong and Thorp, 1999; Prendergast et al., 2020). A recent study on native Australian bees also found that different sexes of bee were sampled differently depending on the method (Prendergast et al., 2020). Thus, a similar difference in preference could exist for flower shape, although the similar confidence intervals for the respective bees in the current study does not suggest sex-based differences for spatial preferences. Despite these methodological limitations, the clear and consistent evidence of different bee species preferring certain flower photographs that are most commonly pollinated by insects (Howard et al., 2019d) is a topic warranting further investigation with more species in different countries around the world. Furthermore, an interesting comparison for future work to examine would be the floral preferences (colour, scent, shape and size) of specialist versus generalist bees. In the current study, we examined the floral shape preferences of two native generalist species which may be visiting a wide array of flowers; however, the same preference may not exist for specialist bee species adapted to visiting just one flower morphology.

Non-eusocial species of bees are comparatively understudied in controlled decision-making and preference experiments despite their abundance and identification as important pollinators of crops (Delaplane et al., 2000; Heard, 1994; Karasiński, 2018). With a growing interest in non-eusocial bees as alternative pollinators to managed eusocial species, the current study provides a successful new method for testing floral preferences of wild non-eusocial bees. As discussed above, non-eusocial species of native bees are more challenging to capture and test than eusocial, hive-living bees such as *A. mellifera*, *B. terrestris* and *T. carbonaria*; thus, this study serves as a bridge to conducting further decision-making experiments on solitary and other non-eusocial bees.

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