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Male tree weta are attracted to cuticular scent cues but do not discriminate according to sex or among two closely related species

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Abstract

Recognition of conspecifics is an essential precursor of successful mating. Where related species coexist, species discrimination might be important, but because related species are similar, species signal recognition may actually be low. Chemical cues such as cuticular hydrocarbons (CHCs) are frequently used by insects to identify suitable sexual partners. We predicted that New Zealand tree weta (*Hemideina* spp.), a genus of nocturnal ensiferan Orthoptera that live both allopatrically and sympatrically, use chemical signals from either frass or CHCs to find mates. In a series of six laboratory trials using both *H. thoracica* and *H. crassidens*, we found that male tree weta, but not female tree weta, occupied cavities primed with female cuticular cues more often than cavities without. However, males did not discriminate between chemical cues of male and female conspecifics, or between conspecifics and heterospecifics. In field trials, tree weta did not occupy artificial cavities primed with either female frass or female cuticular cues more often than unscented cavities. However, in both trials weta preferentially returned to cavities that had already been occupied earlier in the trials. A final field trial confirmed the presence of mixed species harems during the mating season in one region of sympatry. Our results suggest that selection on sex and species specific chemical cues that could be used to find conspecific mates is weak. Mixed species aggregations suggest that identification of conspecific mating cues has not evolved to be species specific. We infer that for male tree weta, the cost of mating with heterospecifics is likely less than not mating at all.

KEYWORDS
cuticular hydrocarbons, hybridization, Orthoptera, reproductive interference, signal, species recognition

1 | INTRODUCTION

Finding a suitable mate can be difficult, and the coexistence of closely related species can lead to problems of recognition. Multiple cues and signal modalities contribute to mate recognition, and behavioral isolation among species (Candolin, 2003; Hebets & Papaj, 2005; Jennions & Petrie, 1997; Johnstone, 1996; Mullen, Mendelsen, Schal, & Shaw, 2007), and visual, acoustic, and chemical cues are frequently employed (see Kortet & Hedrick, 2005). In many insects (including Orthopterans), chemical compounds facilitate kin, sex, and species recognition and thus courtship and mating behavior (e.g., Nagamoto, Aonuma, & Hisada, 2005; Phelan, 1997; Ryan & Sakaluk, 2009; Simmons, 1990; Tregenza & Wedell, 1997), and the same mechanism of recognition can be used in both sexual isolation of species ("species recognition") and in mate recognition within a species. Animal signaling may be species specific (e.g., Tobias & Seddon, 2009; West-Eberhard, 1983), but this is not always the case, as in flour beetles (Serrano, Castro, Toro, & Lopez-Fanjul, 2000). Divergent evolution of signaling systems may not always occur in closely related species, and where these species come into contact, signal confusion can result.

Reproductive interference caused by incomplete species recognition systems can lead to substantial fitness costs if individuals attempt to breed with another species (Grether, Losin, Anderson, & Okamoto, 2009; Gröning & Hochkirch, 2008). Misdirected courtship and heterospecific rivalry are just two types of interference that result in wasted time, energy, nutrients, and gametes. If fitness costs are high (for example, in sexually dimorphic species where males fight using large weapons), these costs may act as long-term evolutionary drivers of species specific recognition mechanisms. Infertility, low fitness of hybrids and lack of offspring are all costly outcomes of incomplete species recognition during mating. However, fitness costs may be asymmetric between species, and reproductive interference can result in the displacement of one species by another (e.g., Gröning, Lücke, Finger, & Hochkirch, 2007; Hochkirch, Gröning, & Bücker, 2007).

Interference caused by the overlap of signal channels between species is more likely in related taxa because of anatomical constraints that have an evolutionary basis (de Kort & ten Cate, 2001). Thus, for example, historically allopatric species that are evolutionarily related with recent or narrow range overlap may lack signal divergence (de Kort, den Hartog, & ten Cate, 2002). If the fitness costs of mating or attempting to mate with other closely related species are low, then sympatric lineages may demonstrate low recognition specificity. If, however, fitness costs are high, as might occur if males invest in costly weaponry, then evolutionary selection supporting signal divergence might occur, and males might also be better than females at discriminating. On the other hand, if females invest more in offspring (for example, where males contribute little in the way of nuptial gifts, and hybrid offspring are infertile), then selection may be stronger on female discrimination. Here, we examine signal specificity of chemical cues in two predominantly allopatric orthopteran lineages that do not sing, where competitive exclusion has been inferred to explain their limited range overlap (Bulgarella, Trewick, Minards, Jacobson, & Morgan-Richards, 2014).

Fatty acid-derived hydrocarbons that occur on the surface of the insect epicuticle (CHCs) to prevent desiccation (Howard & Blomquist, 2005; Weddle et al., 2013) have been well documented as mate recognition cues in some cricket species (e.g., Thomas & Simmons, 2008; Tregenza & Wedell, 1997). Cuticular hydrocarbons are chemically stable and low in volatility, and can be excellent recognition cues at close proximity (Howard & Blomquist, 2005). Wide variation in CHCs has been documented at species and population levels for crickets (Mullen et al., 2007), within and between the sexes (Mullen et al., 2007; Thomas & Simmons, 2008; Tregenza & Wedell, 1997; Warthen & Uebel, 1980), although few studies offer experimental evidence that CHCs are used as mate recognition cues. In one genus of New Zealand ensiferans (*Hemideina* spp., Orthoptera, Anostostomatidae, known locally as tree weta), Gibbs (1998) proposed that tree weta most likely communicate using pheromones as they are nocturnal and sound production is limited to stridulation with no detected species differentiation (Field, 2001). We might therefore expect that chemical cues are critical to species and mate recognition in this genus. Volatiles have previously been recorded from fecal pellets (Guignon, 2005), but their role in species or sex recognition is unknown.

New Zealand ensiferans provide an excellent opportunity to examine signal recognition in phylogenetically similar species that have regions of sympatry (Bulgarella et al., 2014; Trewick & Morgan-Richards, 1995). The Auckland tree weta *Hemideina thoracica* and the Wellington tree weta *Hemideina crassidens* are morphologically similar species that are generally allopatric, but sympatric at the edge of their ranges. Evidence suggests that *H. thoracica* has expanded its range southward during the current interglacial, displacing its close relative *H. crassidens* (Bulgarella et al., 2014). Because of these geographical shifts, species recognition may be incomplete as selection for assortative mating exists only where populations are sympatric. Tree weta males have enlarged, costly weaponry that they use to fight other males and gain, or maintain, access to cavities with females in polygynandrous mating systems (Kelly, 2006b). These males also produce very reduced spermatophylaces (nutritious gifts that contain a spermatophore) compared to other Ensifera (Field & Jarman, 2001). Male and female recognition may therefore have different costs and selection pressures. In our investigation of tree weta signal recognition, we first predicted that recognition likely relies on chemical cues such as CHCs. We predicted that because of the evolutionary relatedness of these species, and ongoing range expansion of *H. thoracica*, shared signal channels and unreliable species discrimination was likely but that discrimination might be asymmetric between the sexes and the species if fitness costs are asymmetric. We predicted that asymmetric sexual and species recognition would lead to choice differences when male and female tree weta were presented with cavities in both laboratory and field experiments that were either unscented, or "scented" with either cuticular or fecal (frass) chemical cues. We hypothesized that females would show no preference between female-scented and unscented cavities, whereas males would occupy female-scented cavities in preference to either unscented or male-scented cavities.

2 | MATERIALS AND METHODS

2.1 | Focal species

Laboratory and field trials used two species of tree weta that occur both sympatrically and allopatrically in New Zealand. The Auckland (northern) tree weta *H. thoracica* is distributed in the central and northern part of the North Island of New Zealand including all three field sites (Morgan-Richards, Trewick, & Wallis, 2000; Figure 1). The Wellington tree weta *H. crassidens* has a more southerly distribution, but the two species have many similarities in diet, growth and mating system (Kelly, 2006a,b; Minards, Trewick, Godfrey, & Morgan-Richards, 2014; Wehi & Hicks, 2010). At Turitea Reserve, *H. thoracica* and *H. crassidens* are sympatric, and both are close to their current geographical limits.

Hemideina spp. (tree weta) are sexually dimorphic, polygynandrous Orthoptera, with seven species in this endemic New Zealand genus.

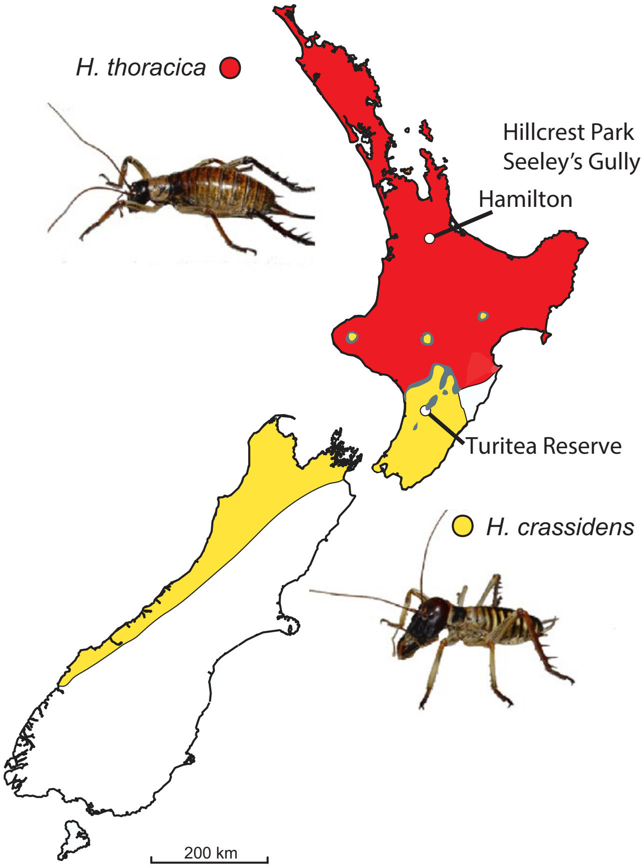

FIGURE 1 The distribution of two New Zealand tree weta species, *Hemideina thoracica* and *Hemideina crassidens* (after Bulgarella et al., 2014), showing the location of forest field sites in North Island that were used to study the role of scent when *H. thoracica* and *H. crassidens* find mates. [Colour figure can be viewed at wileyonlinelibrary.com]

Tree weta are large bodied and flightless (approximately 2–5 g adult weight), and feed at night before finding tree cavities in which to rest diurnally. Cavity loyalty is poorly understood, as males and females may move between different cavities over the course of the summer mating season. Nonetheless, *H. crassidens* and *H. thoracica* adults may return to the same shared cavity over consecutive nights (Wehi, Jorgensen, & Morgan-Richards, 2013), and mating frequently occurs in cavities (Field & Jarman, 2001). Adult tree weta males use their mandibles to fight other males for access to female-occupied cavities (*H. crassidens*, Kelly, 2006b; Moller, 1985). However, male spermatophore investment is small compared to other orthopterans, and females are likely to mate with more than one male in the mating season (Kelly, 2006a).

2.2 | Captive tree weta maintenance

Hemideina thoracica and *H. crassidens* adult individuals of both sexes were caught from sympatric wild populations around Turitea Reserve and maintained singly in captivity in 2-L plastic containers with wire mesh lid to allow light and air circulation. We provided fresh *Melicytus ramiflorus* and *Coprosma robusta* leaves as food (replaced weekly), damp paper, and a daytime refuge cavity made from the hollowed out stem of a harakeke (*Phormium tenax*) flower stalk. We have previously found these harakeke stalk cavities are suitable for diurnal occupation by captive weta (e.g., Wehi, Raubenheimer, & Morgan-Richards, 2013). Weta were maintained in a temperature controlled room at 16°C with a light: dark cycle of 14:10 hr. All weta were weighed prior to the experiments (±0.001 g). Weta were released after laboratory experiments were completed.

2.3 | Captive trial protocols

All captive trials using chemical cuticular cues took place during the Austral summer and autumn in January–April, when *H. thoracica* form harems (Wehi, Jorgensen, et al., 2013). To collect chemical cuticular cues from adult weta, we inserted a circle of filter paper so that it lined the daytime refuge cavity of a mature adult female or male for 48 hr (method modified from Rantala, Jokinen, Kortet, Vainikka, & Suhonen, 2002). Although this method does not optimally extract cuticular lipids, it provides a useful proxy for the odor trails that tree weta might sense in natural cavities, without killing experimental animals. If the selected individual did not use the cavity during both diurnal rest periods, the filter paper was discarded and disks with frass were also discarded. Filter paper disks were removed from the cavities in the evening immediately prior to night time activity. We did this so that the age of the chemical cue would not affect tree weta preferences, and chemical cuticular age would mimic the time period when tree weta were active overnight. The disks were inserted into fresh, unused cavities as a lining, for use in the laboratory experiment. Experimental cavities with a filter paper disk that had previously lined a weta refuge cavity (as above), and hence was impregnated with cuticular cues, are termed "scented" cavities, whereas cavities with a filter disk that had not previously lined a weta refuge are termed "unscented" or control cavities.

In the initial experimental trials, one unscented cavity and one scented cavity of similar dimensions (made from harakeke flower stalks as above) were placed in a test arena, with alternate positioning of the scented cavity on the left or right so that directional bias was not introduced. In later trials to test whether male preference was for cuticular cues per se or specific female cuticular cues, we used a choice test with two scented cavities consisting of (i) a conspecific male and (ii) a conspecific female scent, and a second choice test with (i) a conspecific female and (ii) an other-species female scent. Presentation order of these trials was randomized. Fresh, unused cavities lined with the appropriate filter paper disk were used for each trial.

Trials were conducted in a square, glass-sided arena measuring 23 × 23 × 45 cm with a wire mesh cover. Tree weta were moved at the start of their normal nocturnal phase and tested singly in an arena. Between one and four concurrent trials were run each night, with all arena visually isolated from each other. For each trial, two fresh *C. robusta* leaves were placed centrally in the arena prior to the weta being added, so that food was available for nocturnally foraging weta. Each adult male or female weta was released into the arena at a marked point equidistant from the two available cavities that were placed in close proximity to each other. The individual used to scent the filter paper always differed from the individual used in the experimental trial. Trials were scored at the end of the dark cycle by recording the position of the weta in the arena. Individuals that were inside or immediately adjacent to (<1 cm) a cavity were deemed to have made a "choice." Weta that were elsewhere in the arena were deemed to have made no choice and excluded from analysis (see Appendix 1 for numbers of no choice trials). After each trial, arenas were washed and wiped with 95% EtOH to remove any remaining chemical cues. Each individual weta was used in a single choice experiment.

2.4 | Field trials

We conducted two field experiments to test whether the presence of olfactory chemical cues was an attractant for other weta. Field experiments took place during autumn, when tree weta are sexually active.

2.5 | Field sites

Field trials were conducted at three sites in the North Island, New Zealand. Two of the sites, Hillcrest Park (37°47′46″S, 175°19′18″E; 50 m a.s.l.) and Seeley's Gully (37°47′16″S, 175°17′34″E; 40 m.a.s.l.), are urban forest patches 1.5–2 ha, in Hamilton city (Figure 1), and both are inhabited by *H. thoracica*. Hillcrest Park is remnant lowland forest dominated by mature *Dacrydium dacrydiodes* (kahikatea) >100 years old and 20–25 m tall, with a sparse undergrowth. Twenty of the mature kahikatea trees were randomly selected within Hillcrest Park as host trees for artificial refuges. Seeley's Gully is a mixed broadleaf and podocarp forest remnant, in which 18 mature trees were randomly selected. The third field site at Turitea Reserve (40°25′49.97″S, 175°39′44.12″E;150 m.a.s.l.) is a small, approximately 2-ha section of a managed 3,500-ha reserve in the Manawatu region where both *H. thoracica* and *H. crassidens* are found. The 18 mature, planted *Pinus radiata* trees selected as host trees for artificial refuges were part of the dominant canopy, above the native tree undergrowth. In experiment 1, we used Hillcrest Park to test female *H. thoracica* frass as a potential chemical attractant. In experiment 2, we used all three field sites, but dropped Hillcrest Park from the analysis as only one tree weta was detected over 10 nights.

2.5.1 | Field trial 1

We erected forty artificial refuges, each with two cavities, and constructed from aged totara timbers (*Podocarpus totara*), on 20 marked, mature kahikatea (*Dacrydium dacrydioides*) trees in Hillcrest Park, Hamilton, in late summer. We attached two refuges, one scented and one unscented, to each tree at a standardized height (2.5 m). Refuge direction on the side of the trunk was randomized. Cavity construction mimicked the naturally occurring cavities on tree branches and trunks within which weta rest diurnally, with two unlinked but same sized cavities in each artificial refuge. Each cavity had space to accommodate approximately six adult tree weta. The two cavities in each refuge were subjected to the same treatment, so that effects between treatments occurred at the level of the refuges. Our monitored cavities did not contain frass during or after weta occupancy.

We captured eight adult females from this population during the 2 weeks prior to the experiment and held them in captivity overnight, before later release at their capture sites. Frass produced was frozen immediately after collection in a sealed container. We created a treatment spray by thawing the frass and constituting a mixture, immediately before use, which had a liquid composition of 95% water mixed with 5% female frass. A control spray consisted solely of water. Although female tree weta appear to be sexually receptive throughout the summer and late autumn, we do not have any information about female refractory periods in this genus. Therefore, we combined frass from all eight females to ensure a consistent scent that had a high probability of including any appropriate frass chemical cues.

Refuges were sprayed immediately after placement on site with either the treatment or control spray (day 1). We sprayed both cavity entrance holes of 20 refuges with the treatment frass and water mixture, and the cavity entrance holes of 20 refuges with a water control only. Each tree thus had one treated refuge and one control refuge. After heavy rain on day 3, these treatments were repeated with fresh spray, made up from the frass of the same eight females. We recorded the number and sex of tree weta present in each refuge daily for the first 10 days after refuge attachment to determine the effects of female frass chemical cues on occupancy, as we considered this period most likely to show a difference if one was present, while the scent was still relatively fresh.

2.5.2 | Field trial 2

In trial 2, we erected artificial refuges at Hillcrest Park, Turitea, and Seeley's Gully in late summer 2011, 2012, and 2014, respectively. We used female cuticular scent as a pheromone attractant at all three locations identified above. In this experiment, all refuges had a single cavity that had not previously been inhabited. To achieve pheromone priming, one adult female *H. thoracica* inhabited a cavity for 2 days in the laboratory, prior to attachment of the cavity to a tree on site. The female was removed immediately prior to attachment, in the early evening immediately before tree weta activity began. Unscented refuges were used as is, with no prior inhabitants. Attachment and randomization protocols were the same as in field trial 1, with two refuges erected on each tree (n = 18 trees each at Hillcrest Park and Turitea, and n = 22 trees at Seeley's Gully; n = 116 refuges in total across the three sites). One of the refuges on each tree was primed with female cuticular scent, and the other was not. Tree weta occupants were counted and sexed in each refuge for 10 consecutive days following refuge attachment.

2.5.3 | Field trial 3

To determine whether mixed species harems were forming in the area of sympatry at Turitea Reserve, we monitored 40 artificial single-cavity refuges for tree weta occupancy weekly over a 6-week period in early autumn 2011 and recorded species, sex, and life stage for all tree weta occupying the cavities. All tree weta were marked with nail polish on their pronotum during monitoring, to ensure individual combinations were not counted more than once. A harem was defined as a minimum of one adult male cohabiting with a minimum of one adult female.

The data allow us to determine for each female whether she was cohabiting with a conspecific or heterospecific male. We compared the recorded female cohabiting combinations with those expected by a random distribution of the two species, generating expected values as for the Hardy–Weinberg equation ($p^2 + 2pq + q^2$) based on the frequency of individuals from each species sampled at the site, using a chi-squared test.

We also scored the harems themselves to determine the overall number of mixed and conspecific harems. If a harem included, for example, a male of species A and females from species A and species B, the harem was recorded as both mixed species and same species as two potential combinations were present. In addition, if a new unmarked individual joined a previously marked individual, this was recorded as a new harem, with the new addition determining whether the new was recorded as a same species or mixed species harem.

2.6 | Data analysis

All statistical exploration and analysis was carried out in R (R Core Team, 2015). For the laboratory experiments, we performed chi-squared tests to determine whether tree weta were preferentially attracted to cavities with cuticular chemical cues and included all trials with recorded outcomes. In some cases, the number of trials was small, and we therefore used Monte Carlo simulations to estimate p values. In addition, because we captured different numbers of adult weta of the two species from the wild, not all potential tests of scent discrimination were possible. For this reason, we did not test female *H. crassidens* in scent trials and we did not test male *H. thoracica* with a choice of conspecific male and female scent. For field trials 1 and 2, we selected a data set from day 3 to 10 prior to analysis to compare occupancy of scented and unscented refuges. This data set was a compromise between the length of time we estimated that the pheromones might remain on refuges in the wild (including some rainy conditions) and that required for weta occupancy numbers that would allow robust comparisons. Because most of the cavities were occupied by a tree weta for consecutive nights after initial occupation, we considered it highly likely that the same weta was being recounted in the data in many cases. We therefore examined "initial occupation data" that included only the first night of a continuous occupation (i.e., where a cavity was occupied for consecutive nights) for a cavity. If a break in occupation occurred, the next occupancy was treated as new, rather than a continuation of the previous occupancy.

In order to determine whether scent affected initial cavity occupancy, we fitted generalized linear mixed models with Poisson distributed errors to the daily counts of the number of weta occupying cavities in lme4 (Bates, Maechler, Bolker, & Walker, 2015). We included the scent treatment, weta sex, whether the cavity had been previously occupied during the period of the trial, and two-way interactions between sex and the other variables, as fixed effects. These components were used to test for a sex-specific response to the scent treatments, account for occupancy during the trial and determine whether the response to cavities naturally occupied during the trial was sex specific. Site (cuticle trial only), tree, refuge and cavity (frass trial only) were included as random intercept terms to allow for non-independence of the observations in space and time. The absence of over-dispersion was confirmed by comparing the base model with a model which also included an observation level random effect (Browne, Subramanian, Jones, & Goldstein, 2005). The statistical significance of the fixed effects was determined by simulating from the posterior distributions of the parameter estimates and calculating 95% highest posterior density intervals (HPDI) using the packages coda (Plummer, Best, Cowles, & Vines, 2006) and arm (Gelman et al., 2015).

3 | RESULTS

3.1 | Laboratory trials

Hemideina thoracica males demonstrated a strong preference for cavities primed with female cuticular pheromones when offered a choice of female-scented or unscented cavities ($N = 13$ trials, $N = 12$ males chose scented cavities; $\chi^2 = 9.31$, $p = .003$; Figure 2). In contrast, *H. thoracica* females did not demonstrate a preference for cavities primed with female scent (putative-CHCs) when offered the same choice ($N = 20$ trials, $\chi^2 = 0.2$, $p = .8$). Instead, they chose unscented and scented cavities in almost equal numbers ($N = 9$ and $N = 11$, respectively).

Hemideina crassidens males showed a similar result to the *H. thoracica* males when offered a choice of cavities that were either scented with conspecific female cuticular cues or unscented. Seven of the eight males tested chose a scented cavity ($N = 8$ trials, $\chi^2 = 5.44$, $p < .025$). In a further trial when *H. crassidens* males were offered a choice of cavities impregnated with either conspecific male or conspecific female cuticular pheromones, however, no preference for female pheromones was revealed ($N = 15$ trials, $N = 7$ and $N = 8$, respectively; $\chi^2 = 0.07$, $p = 1.0$). Due to low number of *H. thoracica* males, we did not replicate this trial.

In the mixed species experiments, when *H. thoracica* males were tested with a choice of conspecific or heterospecific female-scented cavities, no preference for conspecific females was revealed ($N = 21$ trials, $N = 12$ conspecific choices; $\chi^2 = 0.43$, $p = .66$, Figure 3). This pattern was repeated in *H. crassidens* ($N = 31$ trials, $N = 16$ conspecific choices; $\chi^2 = 0.03$, $p = 1.0$, Figure 3). Appendix 1 summarizes all trials and respective sample size numbers, including weta who made no choice.

3.2 | Field trial 1: Female frass as a pheromone attractant

At least 18 female and eight male unique weta occupied cavities during the 10-day period. There was no effect of the scented treatment on initial cavity occupancy (arrivals) either overall or dependent on

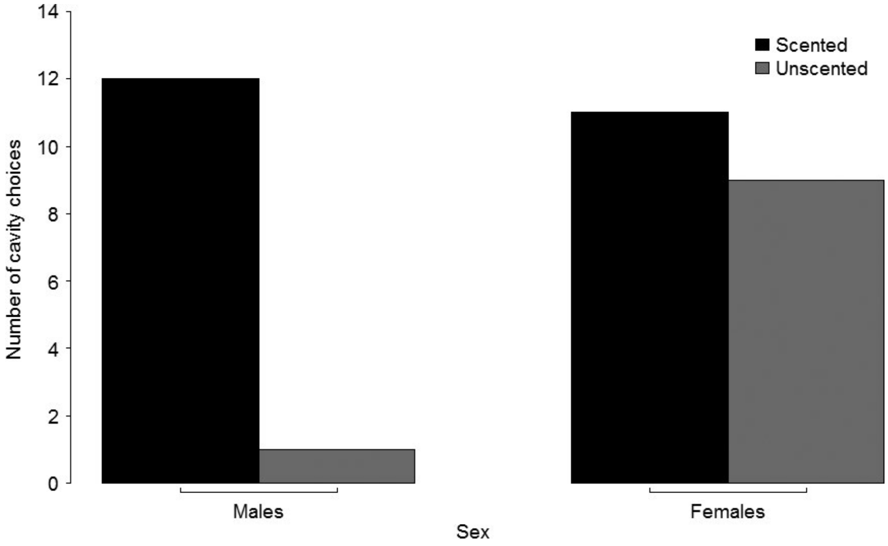

FIGURE 2 Adult *Hemideina thoracica* males occupy refuge cavities primed with conspecific female cuticular cues more often than unscented cavities in laboratory choice experiments. Adult females do not discriminate between cavities with female cuticular cues, and unscented cavities, under the same conditions

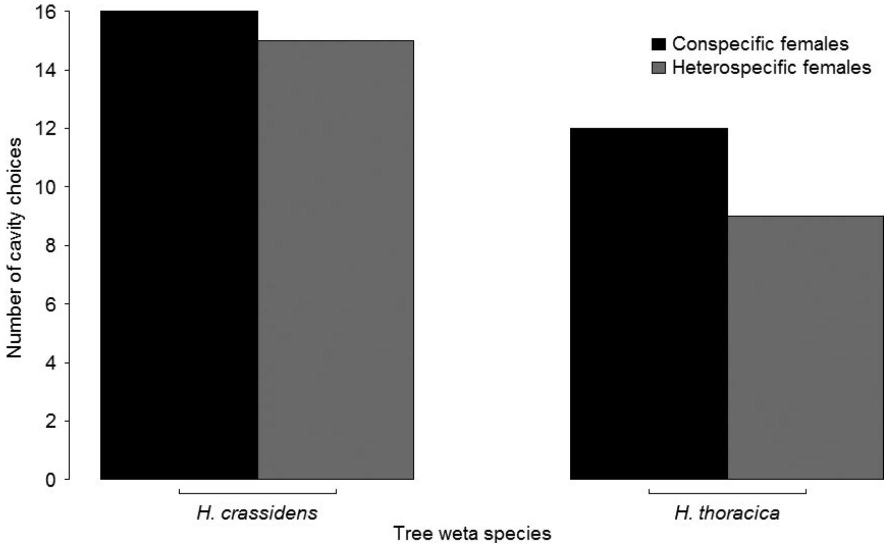

FIGURE 3 Adult male *Hemideina thoracica* and *Hemideina crassidens* did not discriminate between refuge cavities imbued with cuticular cues from females of their own, and females from another tree weta species in a laboratory choice experiment

sex ($p > .05$). Cavities that were occupied earlier in the trial were more likely to contain weta on subsequent days than previously unoccupied cavities, whether the same weta or one weta leaving and another weta arriving (0.05 vs. 0.29 weta/day; parameter estimate [95% HPDI]: 1.83 [1.33–2.32]), but this was not dependent on sex ($p > .05$).

3.3 | Field trial 2: Female cuticular scent as an attractant

Unexpectedly, we did not detect any significant effect of the exposure to cuticular scents on weta occupancy. Overall, there were significantly fewer males occupying cavities than females ($N = 5$ vs. $N = 11$ first night occupancy) 0.035 vs. 0.055 weta/day; parameter estimate [95% HPDI]:−1.21 [−2.47 to −0.189]). Although there was no effect of cuticular scent from the experimental procedure (either overall or dependent on sex [all $p > .05$]), previously occupied cavities were more likely to be occupied on subsequent occasions than previously unoccupied cavities (0.41 vs. 0.01 weta/day; parameter estimate [95% HPDI]: 3.46 [2.81–4.12]). This effect was not sex specific ($p > .05$).

3.4 | Field trial 3: Species aggregations in a region of sympatry

We observed adult male tree weta in the wild sharing artificial cavities with between one and four adult females. We recorded 47 adult females cohabiting with either a male of the same species (76.6%) or a male of the other species (heterospecific 23.4%; Figure 4). Four harems contained both conspecific and heterospecific females. As well as the tree weta in harems, a further single 60 *H. crassidens* and 20 *H. thoracica* individuals were observed in cavities. *H. crassidens* were therefore numerically dominant at this site, and this is reflected in the harem formation data (Figure 4). Using the frequency of the two species at this site to generate expected values, we tested whether females associated with males randomly with respect to species. We observed 29 harems where *H. crassidens* females aggregated with *H. crassidens* males (25.64 expected), 11 heterospecific harems where females were with males of a different species (18.15 expected), and seven harems where *H. thoracica* females aggregated with *H. thoracica* males (3.21 expected). These observations differ significantly from

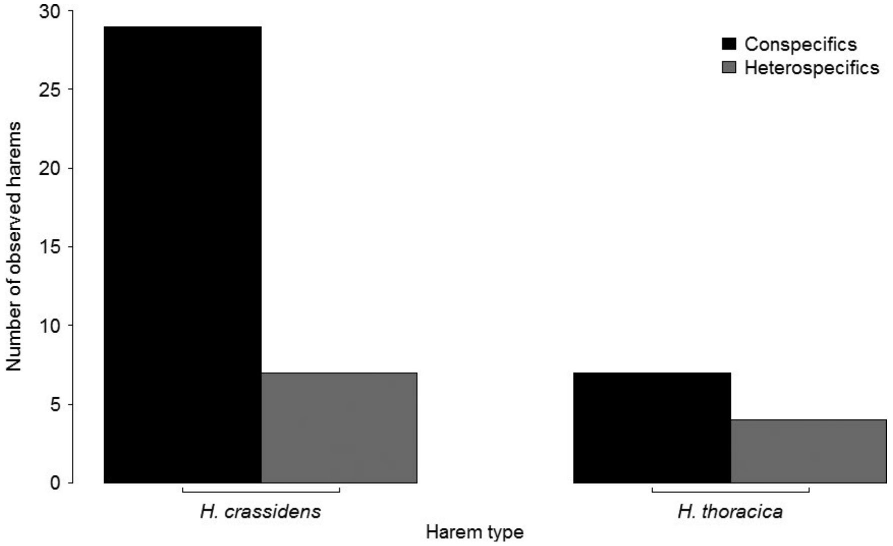

FIGURE 4 Composition of wild tree weta harems recorded during a 6-week period in late summer and autumn, when mating occurs in this polygynandrous genus. Harems are grouped by the species of the male. All individuals recorded were marked on their pronota to prevent double counting the same harem in different time periods. Harems with both conspecific and heterospecific females were counted in both categories, as both females were available for mating by the guarding male

random ($\chi^2_2 = 7.732$; p (2-tailed) = .021). Overall, there were fewer heterospecific aggregations than expected, suggesting at least one sex of one species is discriminating based on species, when sharing daylight refuge cavities.

4 | DISCUSSION

Both sex and species signal recognition appear incomplete in the two species of New Zealand ensiferans tested here, consistent with predictions of signal confusion in closely related and morphologically similar species that have a recent and narrow region of sympatry (Gröning & Hochkirch, 2008). Initial laboratory trials showed that cuticular pheromones appear to be used as a cue for cavity selection by male tree weta of both species, but not females (Figure 2), in agreement with predictions that signal recognition may be stronger where male–male competition limits female choice. In both *Hemideina* species adult males invest heavily in head weaponry and fight other males to increase their access to females. That is, fitness costs might lead to selection on signal recognition, and these putative-CHCs could act as a signal for adult male weta when they occupy cavities during the mating season. These laboratory findings are also consistent with field work showing that male *H. thoracica* occupy cavities previously inhabited by female weta more often than empty cavities (although they avoid staying in cavities with other male weta; Wehi, Jorgensen, et al., 2013). Despite findings, however, signal recognition seems weak overall. In the laboratory experiments, *H. crassidens* males did not discriminate between the chemical cues of male and female conspecifics, and neither *H. crassidens* nor *H. thoracica* males discriminated between the cuticular pheromones of conspecific and heterospecific females (Figure 3). Female *H. thoracica* showed a different behavioral pattern in the laboratory experiments by not preferentially occupying cuticular scented cavities in the laboratory experiments (Figure 2). These findings are nonetheless consistent with *H. thoracica* female occupation patterns observed in the wild (Wehi, Jorgensen, et al., 2013). Our sample sizes were large enough to detect discrimination, as revealed by our trials where male tree weta of both species discriminated between females of their own species and no scent. However, harems in the field were more likely to consist of conspecifics than heterospecifics where both species occur (Figure 4). Reproductive interference caused by signal confusion can result in the displacement of one species by another in regions of sympatry (Gröning et al., 2007; Hochkirch et al., 2007), and these results contribute data that might help explain the observed expansion of *H. thoracica* at the expense of *H. crassidens* at the edge of their ranges.

The field trials conducted here did not clearly identify mate or species specific behavioral patterns in response to cuticular pheromones. It is unclear why this was the case, although it is possible that the wooden refuges used in field trials may not have absorbed female cuticular odor as well as expected. We did detect, however, a high rate of both male and female weta returning to refuges that had previously been occupied during the field trial. This is most likely to reflect cavity loyalty and suggests that tree weta occupy the same cavity for a number of nights.

We found no evidence in the field trials that tree weta frass carries a smell that influences *H. thoracica* use of daytime refuges, despite previous suggestions that frass chemical cues may be used by conspecifics to locate cavities (Bowie, Allen, McCaw, & van Heugten, 2014; Field & Sandlant, 2001; Guignon, 2005). Further testing of frass attractant at different dilutions might be a useful next step to categorically rule out the likelihood of frass as a pheromone attractant. Cavity choice by adult tree weta provides a reasonable proxy for species and sex recognition in a nocturnal, canopy feeding insect where it is difficult to observe mating behavior. Because cavity choices can be observed, and there are frequently many empty cavities in any one population (Wehi, Jorgensen, et al., 2013), we consider that the use of cavities provides important insight into tree weta mate choice.

Harem formation data from the wild provided results that were broadly consistent with the laboratory results, with some mixed species harems occurring that likely resulted from reproductive interference between the species. The evidence suggests signal confusion might lead to erroneous mate choice, based on apparent lack of recognition between conspecifics and heterospecifics. This evidence is compatible with molecular data that indicate tree weta do not sufficiently distinguish intraspecific sexual partners from others of the same genus, resulting in the production of low fertility F_1 hybrids, and the detection of limited gene flow (McKean, Trewick, & Morgan-Richards, 2016; Morgan-Richards, King, & Trewick, 2001; Morgan-Richards & Townsend, 1995). Female F_1 hybrids are sterile and male F_1 hybrids have limited fertility, providing a strong selective force for assortative mating in the region of sympatry (McKean et al., 2016). Our observations of harems in the wild suggest females were more likely to be found cohabiting with their own species of male than with the other species, indicating that some selection on signal recognition may have occurred for females. This aligns with the prediction that fitness costs for erroneous mate choice may be higher in females than males. Studies of female mating behavior and sperm precedence in these two species are needed to understand these interactions.

We predicted that selection on signal recognition might be asymmetric between species, because of the pattern of range overlap, and displacement of *H. crassidens* by *H. thoracica* (Bulgarella et al., 2014). Although the harem data showed more *H. thoracica* females cohabiting with conspecific males than expected by chance, it is unclear which sex makes the choice. Moreover, in another study, most F_1 hybrids examined had *H. crassidens* mothers (McKean et al., 2016), suggesting *H. thoracica* males might be less discerning than *H. crassidens* males, so it may be that a larger sample size is required to detect asymmetry in signal recognition among the species.

The lack of sex-specific discrimination using CHCs by male tree weta can be explained in several ways. First, it is possible, and likely, that males did not discern a difference between the sexes in CHCs, because there was no difference. However, it is also possible that males are unable to discern an existing difference. Cuticular hydrocarbon sexual dimorphism has been identified in a range of species including at least five species of Orthoptera and likely reflects adaptive divergence driven by sexual selection (Thomas & Simmons, 2008, 2009). However, there are many Orthopterans where sex-specific CHCs have not been identified (Thomas & Simmons, 2008), and selection on signal recognition is likely to be weak where phylogenetically related species are allopatric or recently sympatric. Moreover, it could be argued that if it benefits males to identify cavities that are worth defending and that have a high probability of females using them, both male and female odors might indicate the presence of females locally. In this case, there may not be an advantage in identifying sex-specific scent.

In *Hemideina*, evidence for same-sex sexual behavior in captivity includes male–male mounting (M. Morgan-Richards & P. Wehi, pers. obs.), similarly raising the possibility that sex discrimination within the species may be poor (Parker, 1968; Serrano, Castro, Toro, & Lopez-Fanjul, 1991; Serrano et al., 2000 in Burgevin, Friberg, & Maklakov, 2013). Strong selection on male mounting behavior and mating rate, as could occur within *Hemideina* where males attempt to guard female harems, is likely to result in perception errors (Bailey & French, 2012; Burgevin et al., 2013). Poor sex-specific discrimination of cuticular compounds in *Hemideina* is consistent with these behavioral observations.

A third explanation, however, is that male tree weta might discern a difference in male and female cues, but not discriminate behaviorally if the benefits to choosing a cavity based on either male or female cues are unimportant. For example, males might be positively attracted to male chemical cues if mates can be located nearby. Previous field observations suggest that male *H. thoracica* avoid other males during winter and spring, but not during the summer mating season (Wehi, Jorgensen, et al., 2013). Our data cannot rule out the hypothesis that males recognize sex-specific differences in chemical cues, but do not discriminate between these cues when seeking mates. Both intraspecific competition for access to females and potential male "sneaker" behavior have been documented in this genus (Kelly, 2006b, 2008), and harems with more than one male present have been observed in the wild (e.g., Wehi, Jorgensen, et al., 2013). Choosing cavities based on both female and male cuticular cues may therefore lead to increased mating opportunities.

Incomplete species recognition has implications for the coexistence of these closely related species. The findings raise the possibility that mixed species aggregations (with resulting hybrids; McKean et al., 2016) and lack of interspecific mate recognition result from evolutionarily recent and narrow contact of these species. It would, however, be useful to isolate CHCs to make molecular comparisons with other species, as males can detect some scent that has been left behind by weta. Cuticular hydrocarbons evolve rapidly in other species, for example, in a group of rapidly radiating Hawaiian crickets (Mullen et al., 2007) and in *Drosophila serrata* when mate recognition is under selection (Higgie, Chenoweth, & Blows, 2000). As species' ranges continue to shift with climate change, and larger numbers of related species have overlapping ranges, investigating the specificity and origin of chemical cues that assist with locating suitable mates and habitat, both within and among species, will become increasingly important if preservation of existing species is a conservation priority.

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APPENDIX 1

Test weta in laboratory trials. Trial numbers were dependent on capture numbers from the wild and thus vary according to weta availability

Test individuals	Trial scents (first option vs. second option)	Total number of trials	Number of trials where no choice was made	Number of trials where first option from the "Trial scents" column was chosen
Ht males	Ht females vs. no scent	14	1	12
Ht males	Ht females vs. Hc females	27	6	12
Ht females	Ht females vs. no scent	23	3	11
Hc males	Hc females vs. no scent	8	0	7
Hc males	Hc females vs. Ht females	33	2	16
Hc males	Hc females vs. Hc males	20	5	7