BIOLOGY
LETTERS

royalsocietypublishing.org/journal/rsbl

Research

Cite this article: Díaz-Siefer P, Tapia-Gatica J, Martínez-Harms J, Bergmann J, Celis-Diez JL. 2021 A larval aggregation pheromone as foraging cue for insectivorous birds. *Biol. Lett.* **17**: 20210360.
https://doi.org/10.1098/rsbl.2021.0360

Received: 29 June 2021
Accepted: 7 September 2021

Subject Areas:
behaviour, ecology

Keywords:
avian olfaction, insectivorous birds, aggregation pheromone, animal behaviour

Author for correspondence:
Juan L. Celis-Diez
e-mail: juan.celis@pucv.cl

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5627186.

THE ROYAL SOCIETY
PUBLISHING

A larval aggregation pheromone as foraging cue for insectivorous birds

Pablo Díaz-Siefer[1,2], Jaime Tapia-Gatica[2], Jaime Martínez-Harms[3], Jan Bergmann[4] and Juan L. Celis-Diez[2]

[1]Centro Regional de Investigación e Innovación para la Sostenibilidad de la Agricultura y los Territorios Rurales, CERES, Quillota, Chile
[2]Escuela de Agronomía, Pontificia Universidad Católica de Valparaíso, Quillota, Chile
[3]Instituto de Investigaciones Agropecuarias, INIA-La Cruz, La Cruz, Chile
[4]Instituto de Química, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

PD-S, 0000-0001-5104-9830; JM-H, 0000-0001-5753-3893; JB, 0000-0003-3566-1979; JLC-D, 0000-0001-6356-264X

Although birds have traditionally been considered anosmic, increasing evidence indicates that olfaction plays an important role in the foraging behaviours of insectivorous birds. Recent studies have shown that birds can exploit herbivore-induced plant volatiles and sexual pheromones of adult insects to locate their prey. Many insectivorous birds prey on immature insects, providing relevant ecosystem services as pest regulators in natural and agricultural ecosystems. We asked whether birds could rely on chemical cues emitted by the immature stages of insects to prey on them. To address this question, we performed field experiments to evaluate if insectivorous birds can detect the aggregation pheromone produced by the larvae of the carpenter worm, *Chilecomadia valdiviana*. Groups of five artificial larvae were placed in branches of 72 adult trees in a remnant fragment of a sclerophyllous forest in central Chile. Each grouping of larvae contained a rubber septum loaded with either larval pheromone as treatment or solvent alone as control. We found that the number of larvae damaged by bird pecks was significantly higher in groups with dispensers containing the larval extract than in control groups. Our results show that birds can rely on immature insect-derived chemical cues used for larvae aggregation to prey on them.

1. Introduction

Communication through chemical signals is considered the most ancient form of communication among currently living organisms [1]. From bacteria to animals, chemical cues mediate intra- and interspecific interactions between organisms, allowing them to find food or choose their mate while avoiding noxious conditions, predators and pathogens [2–5]. Olfaction is the main sensory system by which animals perceive chemical cues, and its role in the ecology of animals has been extensively documented across species [6].

Birds have traditionally been considered anosmic (i.e. having little or no sense of smell) and dependent on visual and acoustic signals to guide their behaviour. However, since Bang [7] documented the first anatomical study of avian olfactory function, evidence has accumulated supporting the role of olfaction in the behavioural ecology of birds. Olfaction was initially documented for its role in foraging, navigation and partner-specific recognition in seabirds [8–12]. For instance, penguins and Procellariiformes locate their prey through volatile cues produced by phytoplankton when grazed by zooplankton [8,13]. Since those early studies on seabirds, the use of olfaction by birds has been widely documented across taxonomic levels, mediating a broad range of behaviours, including food searching [14,15], mate choice [16–18], kin recognition [19,20], nest localization [21] and predator avoidance [22,23].

Insectivorous birds have also been documented to rely on olfaction to locate their prey. It has been shown that herbivore-induced plant volatiles can guide birds to prey on the insects inducing the emissions of those volatiles [24–26]. Furthermore, a recent study revealed that birds could exploit the pheromones of adult insects to locate their prey. Saavedra & Amo [27] showed that artificial caterpillars in the proximity to dispensers releasing the pheromone of adult females of the winter moth, *Operophtera brumata*, showed more signs of avian predation than controls. Considering that many bird species prey on immature stages of insects and the relevance of this behaviour in pest control [28], we asked whether insectivorous birds can exploit chemical signals emitted by the larvae they prey on.

The larvae of some lepidopteran species use pheromones to form aggregations [29]. For instance, trail pheromones mediate group foraging in *Malacosoma americanum* and *Thaumetopoea pityocampa* [30]. Another example is the larvae of the carpenter worm, *Chilecomadia valdiviana* (Lepidoptera: Cossidae), which produce an abundant volatile secretion that is attractive to conspecifics and that consists of saturated and unsaturated straight-chain acetates and alcohols with (*Z*)-5,13-tetradecadienyl acetate as its main component [31,32]. *Chilecomadia valdiviana* (Philippi) is a moth native to Chile and Argentina. In this species, the eggs are laid in groups of 30–50 at the branch attachment, in small cracks in the trunk or big branches, or beneath the bark. After hatching, the larvae cluster at the site, boring into the wood, where they feed and remain until the end of their larval development, pupating in individual galleries [33]. However, eventually it is possible to observe larvae at the base of trunks and on branches. The larvae of this species feed on the wood of native and cultivated species of trees and bushes [34,35], with an important impact on fruits and wood production [36]. Larvae vary in colour from pink to dark-orange and grow up to 50 mm in length. Based on the increasing evidence that birds use olfaction to locate their prey and considering that the larvae of *C. valdiviana* produce high amounts (up to 10% of the body weight) of secretions, which have a strong characteristic smell to the human nose, we hypothesized that birds could use the larval secretion of *C. valdiviana* as an olfactory cue to locate and prey on the larvae. To test this hypothesis, we performed a field experiment using pheromone-releasing dispensers and artificial larvae, aiming to disentangle the role of chemical cues in the predation of *C. valdiviana* larvae by birds.

2. Methods

The study was conducted within the distribution range of *C. valdiviana*, in an area of *ca* 20 ha (32°53′ S, 71°10′ W) of southern aspect in the foothills of the coastal mountain range of the La Campana-Peñuelas Biosphere Reserve in central Chile. The vegetation type is characterized by evergreen sclerophyllous matorral [37], dominated by the endemic tree species *Crytocarya alba* (Lauraceae), *Peumus boldus*, (Monimiaceae) and *Schinus latifolius* (Anacardiaceae), and the shrub *Retanilla trinervia* (Rhamnaceae). The canopy of these trees represents the habitat of insectivorous birds from different families (see electronic supplementary material, table S1). Among these bird species, the striped woodpecker (electronic supplementary material, figure S1), the Chilean flicker, the thorn-tailed rayadito and the white-throated tree-runner feed mainly on xylophagous larvae or insects present on trunks and branches [38]. Although Chilean forest insectivorous birds have been classified in different habitat-use guilds such as large-tree users, vertical-profile generalists, understorey species and shrub-users, among others [39], an opportunistic foraging strategy has been documented [40]. Thus, insectivorous birds exploit resources actively on different substrates (electronic supplementary material, table S1).

Larvae of *C. valdiviana* were obtained from local fishing supply stores in Valparaíso and were identified according to Olivares & Angulo [35]. Hexane extracts from larvae were obtained as described earlier [31]. Briefly, homogenized larvae of all instars were stirred with hexane overnight. After filtration, drying over $MgSO_4$, and evaporation of the solvent, the resulting extract was used for preparation of the lures. Dispensers were prepared by loading white rubber septa (Sigma-Aldrich, St Louis, MO, USA, catalogue no. Z553905) with 100 µl of a solution containing 1 mg of pure larval extract in hexane (treatment) or with 100 µl of hexane only (control). Based on Saavedra & Amo [27], who used commercially available pheromone dispensers, we used a dose of 1 mg of the larval extract per lure, which is in the range of most commercial pheromone lures used in monitoring for other lepidopterans [36,41].

We carried out a sentinel experiment using artificial larvae as target. For this experiment, artificial larvae ($n = 360$) of approximately the size of a fifth instar larva (length 2.7–3.5 cm) were made with a combination of brown, pink and orange non-toxic plasticine to resemble the natural colour variation of the *C. valdiviana* larva. A total of 177 orange and 183 pink artificial larvae were used for the experiments. The similarity in colour appearance of natural and artificial larvae for birds was evaluated by representing their colours in the avian tetrahedral colour space [42] (see electronic supplementary material, methods and figure S2).

At the experimental site, 72 adult trees from seven different species were randomly chosen, separated from each other by a distance of 49.6 ± 15 m (mean ± s.d.), according to field conditions. Distance between trees was defined based on information available about the foraging range of the Austral thrush, a common native medium-sized bird (weight *ca* 90 g) that inhabits the area [43,44]. Selected distances were similar to those used by Saavedra & Amo [27] for great tit (*Parus major*) and blue tit (*Cyanistes caeruleus*) and were aimed to maximize the chance that different individuals attempt foraging on the larvae models. All trees were georeferenced using a Garmin e-Trex GPS. At each selected tree, five artificial larvae (with the orange and pink morphs arranged randomly) were glued to the tree with cyanoacrylate and disposed in groups on the principal branch (*ca* 1.5 m height above ground) to increase the probability of detecting bird damage in the larvae. In half of the selected trees ($n = 36$), a dispenser loaded with pheromone was placed near the artificial larvae (see electronic supplementary material, figure S3), while the other trees ($n = 36$) were used as a control, where dispensers were treated with solvent only. The dispensers were fixed with a pin to the tree branch (electronic supplementary material, figure S3). The fieldwork was set up in the Austral spring in mid-October 2019 to guarantee the absence of *C. valdiviana* larvae, which could interfere with the experiment with the presence of their pheromones in the environment [34]. Artificial larvae were monitored after two weeks, recording two response variables: (i) the number of trees with at least one larva with damage by birds, such as peck marks, and (ii) the number of larvae with damage at each tree (following [27]). Any damage not attributable to birds was not considered, e.g. damage inflicted by rodents leave clear signs of their incisor teeth on the artificial larvae [44,45].

We used a generalized linear model (GLM) to evaluate the effect of *C. valdiviana* pheromone treatments on the number of trees with at least one larva with damage by birds. A binomial distribution was assumed for this response variable. To evaluate

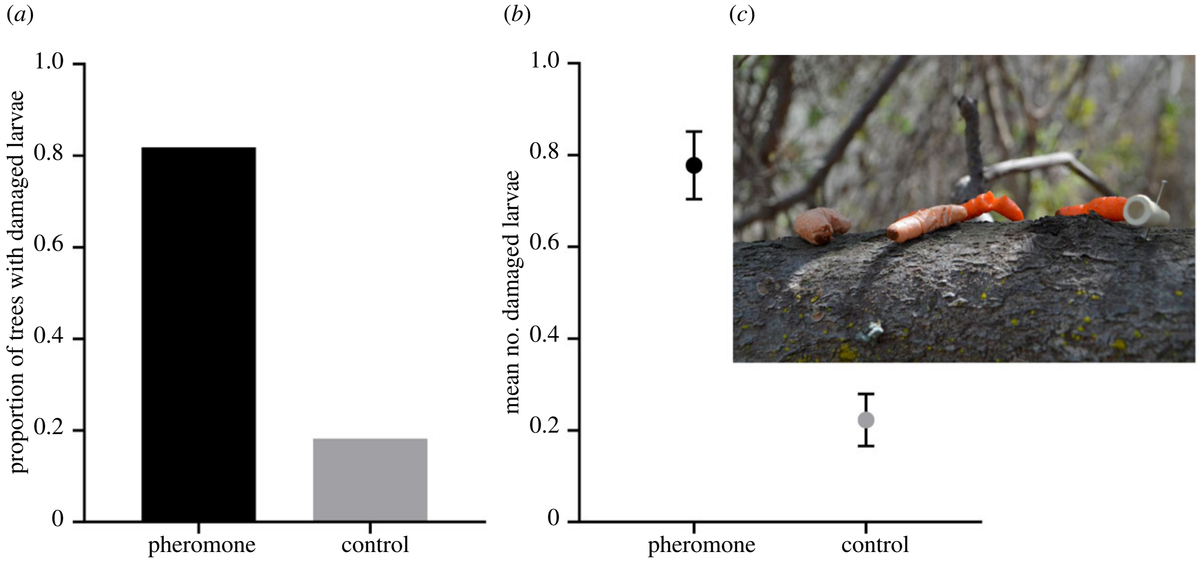

Figure 1. (*a*) Proportion of trees with damaged larvae, (*b*) mean number of damaged larvae in trees with pheromone dispenser and control (error bars in (*b*) indicate one standard error of the mean), and (*c*) example of larvae damaged by bird pecks.

Figure 2. Records of birds pecking artificial larvae. (*a*) The thorn-tailed rayadito, (*b*) the Patagonian tyrant, and (*c*) the Austral blackbird.

the effect of *C. valdiviana* pheromone treatments on the number of larvae with damage at each tree, we used a generalized linear mixed model (GLMM), including treatment as fixed factor and tree species as random factor. For this response variable a Poisson distribution was assumed. Additionally, we discarded spatial autocorrelation of the response variable for each experimental treatment using a Mantel test, considering the spatial location of each tree in the study area (Mantel test = −0.07, $p = 0.84$). Statistical analyses were conducted using the statistical software R v. 4.0.0 [46], using the packages *lme4* [47] and *ade4* [48]. Finally, we installed a camera trap (Bushnell Trophy cam HD) to confirm that birds were responsible for the damage to the larvae and to know which species of insectivorous birds pecked the artificial larvae in our sentinel experiment.

3. Results

We found that artificial caterpillars close to pheromone-emitting dispensers showed significantly more signs of attacks from birds than the control group with unbaited dispensers. From the total number of trees with at least one damaged larva ($n = 22$), 82% ($n = 18$) had dispensers loaded with larval pheromone, while 18% ($n = 4$) had control dispensers (GLM: $Z = 3.32$, $p = 0.000901$; figure 1*a*). The number of larvae showing signs of predation was significantly higher in groups with pheromone-treated dispensers (mean ± s.e. = 0.78 ± 0.07) compared with the control groups (mean ± s.e. = 0.22 ± 0.05, GLMM: $Z = 3.172$, $p = 0.00151$, figure 1*b*), independent of the colour morph (e.g. 19 and 17 damaged larvae from the orange and pink morphs, respectively). In this context, colour variation did not affect the behaviour of birds (see electronic supplementary material, figures S2 and S4).

Based on the photographic records extracted from the trap camera, only birds were recorded to be responsible for the observed damage. The thorn-tailed rayadito (figure 2*a*), the Patagonian tyrant (figure 2*b*) and the Austral blackbird (figure 2*c*) were recorded as the main insectivorous birds that pecked the artificial larvae in our sentinel experiment (figure 2).

4. Discussion

Our results show that insectivorous birds responded positively to the larval aggregation pheromone of the carpenter worm, *C. valdiviana*. The number of trees with at least one artificial larva damaged by bird pecks was more than four times higher in trees with dispensers loaded with pheromones (82%) than with control dispensers (18%). Furthermore, artificial larvae near dispensers with the aggregation pheromone had proportionally more bird pecks than larvae near the control dispenser. These results support previous findings showing that insectivorous birds can use chemical cues to locate and consume their prey

[8,9,11,25,27,49,50]. Considering that many birds prey on immature stages of insects, the capacity to perceive chemical cues emitted by insect larvae would be of great ecological relevance in birds' foraging strategies. To the best of our knowledge, our results provide the first evidence that birds perceive chemical cues emitted by insect larvae and that they can exploit those cues to prey on insects.

The larva of *C. valdiviana* is a polyphagous woodborer native to Argentina and Chile, which mainly feeds on the xylem without killing the tree. The larvae make a single transverse tunnel to the heartwood, turn longitudinally ascending and remain in the tunnel until the pupa stage. Their damage to the tree is almost imperceptible, making it difficult for insectivorous birds to detect the larvae visually. Previous studies suggest that insectivorous birds, such as woodpeckers, use cryptic visual cues to detect woodborer larvae [51,52]. Our results indicate that woodpeckers can also rely on chemical cues emitted by the larvae to detect them. The use of larval aggregation pheromones would reduce woodpeckers' energy expenditure in pecking trees and increase their chances of detecting more prey owing to the increase in the concentration of pheromones in the local environment [29,53].

Many evolutionary advantages have been suggested as to why animals live in groups [54–58], including the selfish herd hypothesis, in which gregarious behaviour diminishes the individual risk of being attacked [59]. It has been proposed that by forming aggregations, insect larvae would increase their foraging efficiency, growth rate and survivorship (see [57,58,60]). According to our results, living in groups would also have detrimental effects, given that the aggregation of larvae would increase the concentration of pheromones in their immediate surroundings, making them more detectable by birds.

While only a few studies have addressed the aggregation behaviours of lepidopteran larvae, the chemical cues mediating such behaviours have received even less attention [61,62]. In addition to studies on *C. valdiviana*, compounds eliciting aggregation have been identified for the larvae of the greater wax moth, *Galleria mellonella* [63], and the codling moth, *Cydia pomonella* [64]. Experiments using real and artificial larvae of the codling moth, a major pest in apple crops, revealed that birds prey on real larvae significantly more than plasticine models [65]. These results are in line with ours in that visual cues alone do not induce larvae predation rates as high as when combined with chemical cues. The increasing amount of evidence supporting the role of olfaction in birds' foraging behaviours, together with the extensive knowledge on chemical communication among insects, suggest that the use chemical cues in the predation of insect larvae by birds might be a widespread phenomenon.

Herbivorous insects are considered one of the main pests in agriculture. Insectivorous birds play an important ecological role in controlling herbivores in natural and agricultural ecosystems [28,66]. For instance, woodpeckers (Picidae) were shown to regulate the population of bark beetles (Curculionidae) in coniferous forest landscapes [67]. Moreover, different studies have demonstrated that insectivorous birds use herbivore-induced plant volatiles as olfactory cues to locate their prey in agricultural ecosystems [25,49]. Within this context, the capacity of birds to detect insect-derived chemical cues, such as sexual or aggregation pheromones, may present an opportunity for the regulation of insect populations in human-modified landscapes. Further studies are needed to understand the mechanisms by which birds perceive insect-derived chemical cues and the impact of avian olfaction on larval predation.

Ethics. This research complies with ethical guidelines by the Pontificia Universidad Católica de Valparaíso under approval no. BIOEPUCV-BA 331-2020.

Data accessibility. Original data and code associated with this manuscript are available in the Figshare repository https://doi.org/10.6084/m9.figshare.15173706.v1 [68] and in the electronic supplementary material.

Authors' contributions. P.D.-S., J.T.-G., J.B. and J.L.C.-D. conceived and designed the study. P.D.-S. and J.T.-G. conducted fieldwork and data acquisition. P.D.-S., J.M.-H. and J.L.C.-D. analysed the data. P.D.-S,. J.T.-G., J.M.-H., J.B. and J.L.C.-D. wrote the manuscript. P.D.-S., J.M.-H., J.B. and J.L.C.-D. edited the manuscript. All the authors approved the final version of the manuscript and agreed to be held accountable for all aspects of the work.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by ANID/PIA/ACT192027 (Chile) to J.L.C.-D. and P.D.-S., ANID/REGIONAL/CERES/R19A1002 (Chile) to P.D.-S., and a PhD scholarship from DEA-PUCV (Chile) to J.T.-G. J.L.C.-D. is Associated Researcher of the Instituto de Ecologia y Biodiversidad (IEB-Chile) funded by ANID/PIA/AFB170008.

Acknowledgements. We thank N. Olmos, F. Lillo and W. Barros for field assistance. We acknowledge F. Fontúrbel for his support in reviewing the language, our children who made the artificial plasticine larvae, and G. Fuster for his permission to use the striped woodpecker photograph. J.M.-H. acknowledges the support of the Ministry of Agriculture, Chile.

References

1. Bradbury JW, Vehrencamp SL. 2011 *Principles of animal communication*, 2nd edn. Sunderland, MA: Sinauer Associates.
2. Grebe TW, Stock J. 1998 Bacterial chemotaxis: the five sensors of a bacterium. *Curr. Biol.* **8**, 154–157. (doi:10.1016/s0960-9822(98)00098-0)
3. Johansson BG, Jones TM. 2007 The role of chemical communication in mate choice. *Biol. Rev.* **82**, 265–289. (doi:10.1111/j.1469-185X.2007.00009.x)
4. Kaupp UB. 2010 Olfactory signalling in vertebrates and insects: differences and commonalities. *Nat. Rev. Neurosci.* **11**, 188–200. (doi:10.1038/nrn2789)
5. Cande J, Prud'homme B, Gompel N. 2013 Smells like evolution: the role of chemoreceptor evolution in behavioral change. *Curr. Opin. Neurobiol.* **23**, 152–158. (doi:10.1016/j.conb.2012.07.008)
6. Rasmussen LEL, Lee TD, Roelofs WL, Zhang A, Daves GD. 1996 Insect pheromone in elephants. *Nature* **379**, 684. (doi:10.1038/379684a0)
7. Bang BG. 1960 Anatomical evidence for olfactory function in some species of birds. *Nature* **188**, 547–549. (doi:10.1038/188547a0)
8. Nevitt GA, Veit RR, Kareiva P. 1995 Dimethyl sulphide as a foraging cue for seabirds. *Nature* **376**, 680–682. (doi:10.1038/376680ao)
9. Cunningham GB, Van Buskirk RW, Bonadonna F, Weimerskirch H, Nevitt GA. 2003 A comparison of the olfactory abilities of three species of procellariiform chicks. *J. Exp. Biol.* **206**, 1615–1620. (doi:10.1242/jeb.00286)
10. Abolaffio M, Reynolds AM, Cecere JG, Paiva VH, Focardi S. 2018 Olfactory-cued navigation in shearwaters: linking movement patterns to mechanisms. *Scient. Rep.* **8**, 11590. (doi:10.1038/s41598-018-29919-0)
11. Bonadonna F, Nevitt GA. 2004 Partner-specific odor recognition in an Antarctic seabird. *Science* **306**, 835. (doi:10.1126/science.1103001)

12. Gagliardo A, Bried J, Lambardi P, Luschi P, Wikelski M, Bonadonna F. 2013 Oceanic navigation in Cory's shearwaters: evidence for a crucial role of olfactory cues for homing after displacement. *J. Exp. Biol.* **216**, 2798–2805. (doi:10.1242/jeb.085738)
13. Amo L, Rodríguez-Gironés MÁ, Barbosa A. 2013 Olfactory detection of dimethyl sulphide in a krill-eating Antarctic penguin. *Mar. Ecol. Prog. Ser.* **474**, 277–285. (doi:10.3354/meps10081)
14. Smith HR, DeGraaf RM, Miller RS. 2002 Exhumation of food by turkey vulture. *J. Raptor Res.* **36**, 144–145.
15. Grigg NP, Krilow JM, Gutierrez-Ibanez C, Wylie DR, Graves GR, Iwaniuk AN. 2017 Anatomical evidence for scent guided foraging in the turkey vulture. *Scient. Rep.* **7**, 17408. (doi:10.1038/s41598-017-17794-0)
16. Amo L, López-Rull I, Pagán I, Macías Garcia C. 2012 Male quality and conspecific scent preferences in the house finch, *Carpodacus mexicanus*. *Anim. Behav.* **84**, 1483–1489. (doi:10.1016/j.anbehav.2012.09.021)
17. Whittaker DJ, Gerlach NM, Soini HA, Novotny MV, Ketterson ED. 2013 Bird odour predicts reproductive success. *Anim. Behav.* **86**, 697–703. (doi:10.1016/j.anbehav.2013.07.025)
18. Caro SP, Balthazart J, Bonadonna F. 2015 The perfume of reproduction in birds: chemosignaling in avian social life. *Horm. Behav.* **68**, 25–42. (doi:10.1016/j.yhbeh.2014.06.001)
19. Krause ET, Krüger O, Kohlmeier P, Caspers BA. 2012 Olfactory kin recognition in a songbird. *Biol. Lett.* **8**, 327–329. (doi:10.1098/rsbl.2011.1093)
20. Caspers BA, Hagelin JC, Paul M, Bock S, Willeke S, Krause ET. 2017 Zebra finch chicks recognize parental scent, and retain chemosensory knowledge of their genetic mother, even after egg cross-fostering. *Scient. Rep.* **7**, 12859. (doi:10.1038/s41598-017-13110-y)
21. Caspers BA, Krause ET. 2011 Odour-based natal nest recognition in the zebra finch (*Taeniopygia guttata*), a colony-breeding songbird. *Biol. Lett.* **7**, 184–186. (doi:10.1098/rsbl.2010.0775)
22. Amo L, Galván I, Tomás G, Sanz JJ. 2008 Predator odour recognition and avoidance in a songbird. *Funct. Ecol.* **22**, 289–293. (doi:10.1111/j.1365-2435.2007.01361.x)
23. Mahr K, Hoi H. 2018 Red-legged partridges perceive the scent of predators and alarm scents of an avian heterospecific. *Anim. Behav.* **144**, 109–114. (doi:10.1016/j.anbehav.2018.08.008)
24. Amo L, Jansen JJ, van Dam NM, Dicke M, Visser ME. 2013 Birds exploit herbivore-induced plant volatiles to locate herbivorous prey. *Ecol. Lett.* **16**, 1348–1355. (doi:10.1111/ele.12177)
25. Hiltpold I, Shriver WG. 2018 Birds bug on indirect plant defenses to locate insect prey. *J. Chem. Ecol.* **44**, 576–579. (doi:10.1007/s10886-018-0962-0)
26. Mrazova A, Sam K, Amo L. 2019 What do we know about birds' use of plant volatile cues in tritrophic interactions? *Curr. Opin. Insect Sci.* **32**, 131–136. (doi:10.1016/j.cois.2019.02.004)
27. Saavedra I, Amo L. 2018 Insectivorous birds eavesdrop on the pheromones of their prey. *PLoS ONE* **13**, e0190415. (doi:10.1371/journal.pone.0190415)
28. Nyffeler M, Şekercioğlu ÇH, Whelan CJ. 2018 Insectivorous birds consume an estimated 400–500 million tons of prey annually. *Sci. Nat.* **105**, 47. (doi:10.1007/s00114-018-1571-z)
29. Duthie B, Gries G, Gries R, Krupke C, Derksen S. 2003 Does pheromone-based aggregation of codling moth larvae help procure future mates? *J. Chem. Ecol.* **29**, 425–436. (doi:10.1023/A:1022690129959)
30. Fitzgerald TD. 1993 Trail and arena marking by caterpillars of *Archips cerasivoranus* (Lepidoptera: Tortricidae). *J. Chem. Ecol.* **19**, 1479–1489. (doi:10.1007/BF00984891)
31. Bergmann J, Lopez K, Buono-Core G. 2007 Identification and synthesis of some fatty acid derivatives from larvae of *Chilecomadia valdiviana* (Lepidoptera: Cossidae). *Nat. Prod. Res.* **21**, 473–480. (doi:10.1080/14786410601129986)
32. Reyes-Garcia L, Flores MF, Vera W, Bergmann J. 2011 Biological activity of the larval secretion of *Chilecomadia valdiviana*. *J. Chem. Ecol.* **37**, 1137–1142. (doi:10.1007/s10886-011-0023-4)
33. Rojas E, Gallardo R. 2004 Familia Cossidae [Family Cossidae]. In *Manual de insectos asociados a maderas en la zona sur de Chile* [Technical manual of wood-associated insects in southern Chile], pp. 45–47. Santiago, Chile: Unidad de Comunicaciones, SAG. [In Spanish.]
34. Angulo AO, Olivares TS. 1991 *Chilecomadia valdiviana* (Philippi) (Lepidoptera: Cossidae) asociado a *Ulmus glabra* Hudson forma pendula (Laud.) Rehder (*Olmo pendula*) en la VIII Región (Concepción, Chile) [*Chilecomadia valdiviana* (Philippi) (Lepidoptera: Cossidae) associated with *Ulmus glabra* Hudson pendula variety (Laud.) Rehder (*Ulmus pendula*) in the VIII region (Concepción, Chile). *Bosque* **12**, 67–68. (doi:10.4206/bosque.1991.v12n1-09) [In Spanish.]
35. Olivares TS, Angulo AO. 1992 *Chilecomadia valdiviana* (Philippi): description of the larva and pupa (Lepidoptera: Cossidae). *Gayana Zool.* **56**, 181–184.
36. Barros-Parada W, Fuentes-Contreras E, Bergmann J, Herrera H, Kinsho T, Miyake Y. 2021 Monitoring *Chilecomadia valdiviana* (Lepidoptera: Cossidae) using sex pheromone-baited traps in apple orchards in Chile. *Insects* **12**, 511. (doi:10.3390/insects12060511)
37. Armesto JJ, Martínez JA. 1978 Slope aspect in the Mediterranean region of Chile. *J. Ecol.* **66**, 881–889. (doi:10.2307/2259301)
38. Jaksic FM, Feisinger P. 1991 Bird assemblages in temperate forests of North and South America: a comparison of diversity, dynamics, guild structure and resource use. *Rev. Chil. Hist. Nat.* **64**, 491–510.
39. Díaz IA, Armesto JJ, Reid S, Sieving KE, Willson MF. 2005 Linking forest structure and composition: Avian diversity in successional forests of Chiloé Island, Chile. *Biol. Conserv.* **123**, 91–101. (doi:10.1016/j.biocon.2004.10.011)
40. Muñoz CE, Ippi S, Celis-Diez JL, Salinas D, Armesto JJ. 2017 Arthropods in the diet of the bird assemblage from a forested rural landscape in northern Chiloé island, Chile: a quantitative study. *Ornitol. Neotrop.* **28**, 191–199.
41. Flores MF, Bergmann J, Ballesteros C, Arraztio D, Curkovic T. 2021 Development of monitoring and mating disruption against the Chilean leafroller *Proeulia auraria* (Lepidoptera: Tortricidae) in orchards. *Insects* **12**, 625. (doi:10.3390/insects12070625)
42. Goldsmith TH. 2011 Optimization, constraint, and history in the evolution of eyes. *Q. Rev. Biol.* **65**, 281–322. (doi:10.1086/416840)
43. Muñoz CE, Undurraga MI, Saratscheff T, Rannou T, Celis-Diez JL. 2018 Diversidad y conocimiento de las aves urbanas por habitantes de Santiago, Chile [Diversity and knowledge of urban birds by inhabitants of Santiago, Chile]. In *Biodiversidad urbana en Chile: estado del arte y los desafíos futuros* [Urban biodiversity in Chile: state of the art and future challenges] (eds J Figueroa, I Lazzoni), pp. 285–315. Santiago, Chile: Ediciones Universidad Central. [In Spanish.]
44. Pérez-Hernández CG, Vergara PM, Saura S, Hernández J. 2015 Do corridors promote connectivity for bird-dispersed trees? The case of *Persea lingue* in Chilean fragmented landscapes. *Landsc. Ecol.* **30**, 77–90. (doi:10.1007/s10980-014-0111-2)
45. González-Gómez PL, Estades CF, Simonetti JA. 2006 Strengthened insectivory in a temperate fragmented forest. *Oecologia* **148**, 137–143. (doi:10.1007/s00442-005-0338-3)
46. R Core Team. 2020 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See https://www.R-project.org/.
47. Bates D, Mächler M, Bolker BM, Walker SC. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–41. (doi:10.18637/jss.v067.i01)
48. Dray S, Dufour A. 2007 The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Soft.* **22**, 1–20. (doi:10.18637/jss.v022.i04)
49. Rubene D, Leidefors M, Ninkovic V, Eggers S, Low M. 2019 Disentangling olfactory and visual information used by field foraging birds. *Ecol. Evol.* **9**, 545–552. (doi:10.1002/ece3.4773)
50. Silva MC, Chibucos M, Munro JB, Daugherty S, Coelho MM, Silva JC. 2020 Signature of adaptive evolution in olfactory receptor genes in Cory's shearwater supports molecular basis for smell in procellariiform seabirds. *Scient. Rep.* **10**, 543. (doi:10.1038/s41598-019-56950-6)
51. O'Daniels ST, Kesler DC, Mihail JD, Webb EB, Werner SJ. 2017 Functional visual sensitivity to ultraviolet wavelengths in the pileated woodpecker (*Dryocopus pileatus*), and its influence on foraging substrate selection. *Physiol. Behav.* **174**, 144–154. (doi:10.1016/j.physbeh.2017.02.041)

52. O'Daniels ST, Kesler DC, Mihail JD, Elisabeth B, Werner SJ. 2018 Visual cues for woodpeckers: light reflectance of decayed wood varies by decay fungus. *Wilson J. Ornithol.* **130**, 200–212. (doi:10.1676/16-171.1)
53. Fitzgerald TD. 2003 Role of trail pheromone in foraging and processionary behavior of pine processionary caterpillars *Thaumetopoea pityocampa*. *J. Chem. Ecol.* **29**, 513–532. (doi:10.1023/A:1022875102682)
54. Rubenstein DI. 1978 On predation, competition, and the advantages of group living. In *Social behavior* (eds PPG Bateson, PH Klopfer), pp. 205–231. Boston, MA: Springer.
55. Rubenstein D, Kealey J. 2010 Cooperation, conflict, and the evolution of complex animal societies. *Nat. Educ. Knowl.* **3**, 78.
56. Chapman CA, Valenta K. 2015 Costs and benefits of group living are neither simple nor linear. *Proc. Natl Acad. Sci. USA* **112**, 14 751–14 752. (doi:10.1073/pnas.1519760112)
57. Denno RF, Benrey B. 1997 Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecol. Entomol.* **22**, 133–141. (doi:10.1046/j.1365-2311.1997.t01-1-00063.x)
58. Clark BR, Faeth SH. 1997 The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecol. Entomol.* **22**, 408–415. (doi:10.1046/j.1365-2311.1997.00091.x)
59. Foster WA, Treherne JE. 1981 Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature* **293**, 466–467. (doi:10.1038/293466a0)
60. Iwakuma T, Morimoto N. 1984 An analysis of larval mortality and development in relation to group size in *Dictyoploca japonica* Butler (Lepidoptera: Saturniidae), with special reference to field populations. *Res. Popul. Ecol. (Kyoto)* **26**, 51–73. (doi:10.1007/BF02515507)
61. Fitzgerald TD, Kelly M, Potter T, Carpenter JE, Rossi F. 2015 Trail following response of larval *Cactoblastis cactorum* to 2-acyl-1,3-cyclohexanediones. *J. Chem. Ecol.* **41**, 409–417. (doi:10.1007/s10886-015-0567-9)
62. Fitzgerald TD, Wolfin M, Young R, Meyer K, Fabozzi E. 2016 Collectively facilitated behavior of the neonate caterpillars of *Cactoblastis cactorum* (Lepidoptera: Pyralidae). *Insects* **7**, 59. (doi:10.3390/insects7040059)
63. Kwadha CA, Mutunga JM, Irungu J, Ongamo G, Ndegwa P, Raina S, Fombong AT. 2019 Decanal as a major component of larval aggregation pheromone of the greater wax moth, *Galleria mellonella*. *J. Appl. Entomol.* **143**, 417–429. (doi:10.1111/jen.12617)
64. Jumean Z, Gries R, Unruh T, Rowland E, Gries G. 2005 Identification of the larval aggregation pheromone of codling moth, *Cydia pomonella*. *J. Chem. Ecol.* **31**, 911–924. (doi:10.1007/s10886-005-3552-x)
65. Peisley RK, Saunders ME, Luck GW. 2016 Cost-benefit trade-offs of bird activity in apple orchards. *PeerJ* **4**, e2179. (doi:10.7717/peerj.2179)
66. Mazia CN, Chaneton EJ, Kitzberger T, Garibaldi LA. 2009 Variable strength of top-down effects in *Nothofagus* forests: bird predation and insect herbivory during an ENSO event. *Austral. Ecol.* **34**, 359–367. (doi:10.1111/j.1442-9993.2009.01933.x)
67. Fayt P, Machmer MM, Steeger C. 2005 Regulation of spruce bark beetles by woodpeckers—a literature review. *For. Ecol. Manag.* **206**, 1–14. (doi:10.1016/j.foreco.2004.10.054)
68. Díaz-Siefer P, Tapia-Gatica J, Martínez-Harms J, Bergmann J, Celis-Diez JL. 2021 Data from: A larval aggregation pheromone as foraging cue for insectivorous birds. FigShare. (doi:10.6084/m9.figshare.15173706.v1)

ELECTRONIC SUPPLEMENTARY MATERIAL

A larval aggregation pheromone as foraging cue for insectivorous birds

Pablo Díaz-Siefer, Jaime Tapia-Gatica, Jaime Martínez-Harms, Jan Bergmann,
Juan L. Celis-Diez

Supplementary Methods:

To evaluate the similarity in appearance of larvae for birds, their colours were represented in the avian tetrahedral colour space [1]. For this, the spectral reflectance of natural and artificial larvae was measured (see figure S2) with a spectrometer (Ocean Optics USD2000, Dunedin, FL, USA) that through an optical fibre collected the light reflected by patches of larvae illuminated through a second fibre by a deuterium-halogen lamp (Avantes AvaLight-DH-S). A white reference standard (Avantes WS-2) was used to calibrate the spectrometer. The spectral reflectance of the larvae was then plotted in a tetrahedral colour space (see figure S4) modelled using the spectral sensitivity of birds' cone type photoreceptors as described by Kelber et al. [2]. Two classes of colour vision have been described in birds, in which the cone sensitive to shorter wavelengths are maximally sensitive to ultraviolet (UVS) or violet (VS) light [3]. Although there is no data about the colour vision of the bird species recorded in our experiments, information about related species indicate that some may have VS while others UVS cone types. For instance, most Passeriformes are thought to have UVS type cones, except for Corvidae, Tyrannidae and Meliphagidae [4]. On the other hand, for woodpeckers the available evidence indicates that they have VS type cones [4]. An avian tetrahedral space was modelled considering the standard avian colour vision system with VS type cones, and the analysis was repeated with a colour vision system with UVS con type cones. The analyses were performed using the PAVO package [5].

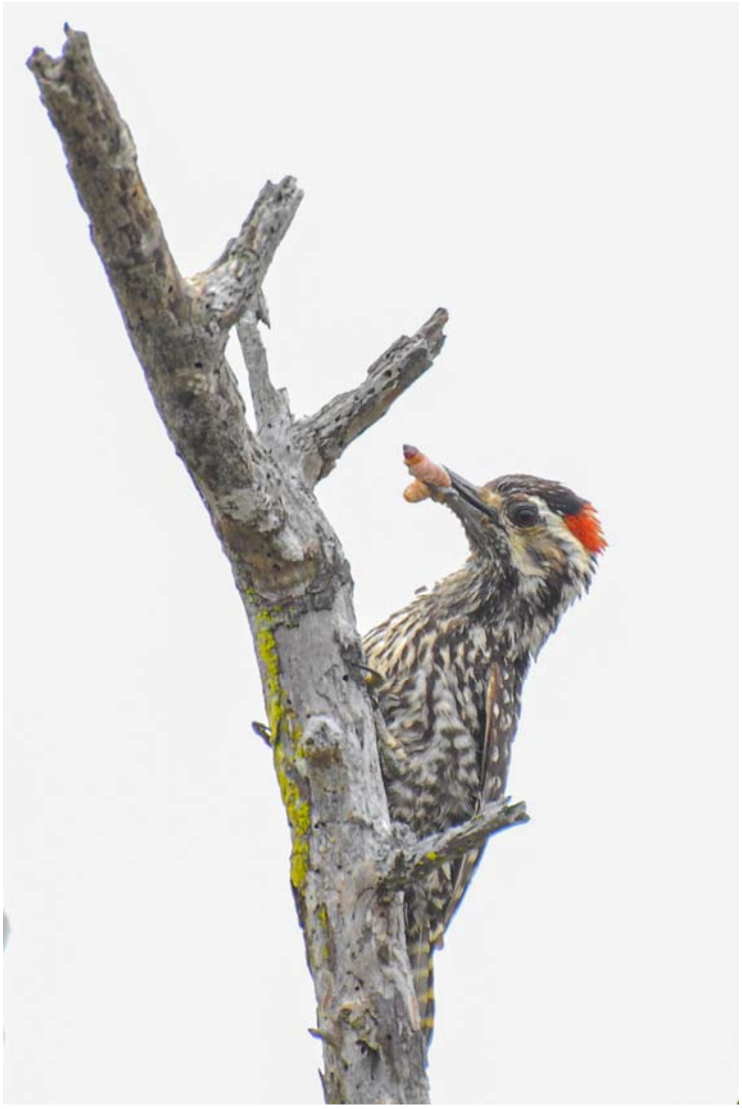

Figure S1. Example of the Striped Woodpecker feeding on a carpenter worm *Chilecomadia valdiviana* larva in central Chile (credit G. Fuster).

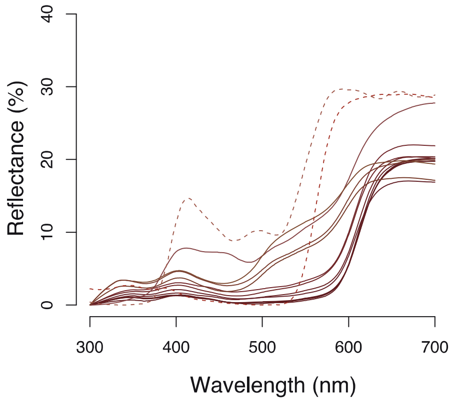

Figure S2. Spectral reflectance of natural larvae of *C. valdiviana* (continuous line) and the artificial larvae used in the experiments (dashed line)**.**

Figure S3. Example of the sentinel experiment using artificial larvae of the carpenter worm *Chilecomadia valdiviana* and the pheromone dispenser.

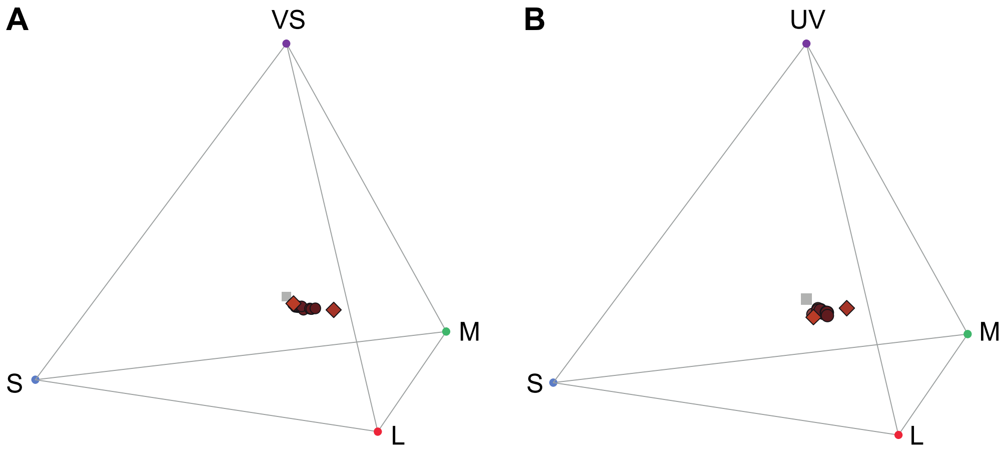

Figure S4. Distribution of colours of natural (circles) and artificial (squares) larvae in the avian tetrahedral space modelled using the photoreceptor spectral sensitivity of species having VS type cones (A) and species having UV type cones (B). Note that the loci occupied by artificial larvae tend to overlap with the loci occupied by natural larvae.

Supplementary Table S1. Feeding behaviour and habitat guild for potential canopy-users insectivorous birds that inhabit the study area [6, 7].

Bird Species	**Common name**	**Order**	**Family**	**Feeding and habitat guild**
Striped Woodpecker	*Dryobates lignarius*	Piciformes	Picidae	Insectivorous bird of the canopy of trees and shrubs. Feed on insects and wood-boring larva, drilling the trunk or branches, and searching beneath the surface of tree bark.
Chilean Flicker	*Colaptes pitius*	Piciformes	Picidae	Insectivorous bird of the canopy of trees, shrubs and sometimes the ground. Feed on insects and wood-boring larva, drilling the trunk or branches, and searching beneath the surface of tree bark.
Thorn-tailed Rayadito	*Aphrastura spinicauda*	Passeriformes	Furnariidae	Insectivorous bird of the canopy of trees. Feed on canopy insects and wood-boring larva searching beneath the surface of tree bark.
Patagonian Tyrant	*Colorhamphus parvirostris*	Passeriformes	Tyrannidae	Insectivorous bird of the canopy of trees. Feed on canopy insects and beneath the surface of tree bark.
White-throated Tree-runner	*Pygarrhichas albogularis*	Passeriformes	Furnariidae	Insectivorous bird of the canopy of trees. Feed on canopy insects and wood-boring larva searching beneath the surface of tree bark.
Plain-mantled Tit-spinetail	*Leptasthenura aegithaloides*	Passeriformes	Furnariidae	Insectivorous bird of the canopy of trees and shrubs. Feed on canopy insects and larva searching beneath the surface of tree bark.
House Wren	*Troglodytes aedon*	Passeriformes	Troglodytidae	Insectivorous bird of the canopy of trees, shrubs and sometimes the ground. Feed on insects.
Austral Blackbird	*Curaeus curaeus*	Passeriformes	Icteridae	Omnivorous bird that feed on insect in the canopy of tree, shrubs and in the ground.
Fire-eyed Diucon	*Xolmis pyrope*	Passeriformes	Tyrannidae	Omnivorous bird that feed on insect in the canopy of tree, shrubs and in the ground
Austral Thrush	*Turdus falcklandii*	Passeriformes	Turdidae	Omnivorous bird that feed on insect in the canopy of tree, shrubs and in the ground
Chilean Mockingbird	*Mimus thenca*	Passeriformes	Mimidae	Omnivorous bird that feed on insect in the canopy of tree, shrubs and in the ground
White-crested Elaenia	*Elaenia albiceps*	Passeriformes	Tyrannidae	Omnivorous bird that feed on insect in the canopy of tree, shrubs and in the ground

Supplementary References:

1. Goldsmith TH. 2011 Optimization, constraint, and history in the evolution of eyes. *Q. Rev. Biol.* **65**, 281–322. (doi:10.1086/416840)
2. Kelber A, Vorobyev M, Osorio D. 2003 Animal colour vision - Behavioural tests and physiological concepts. *Biol. Rev. Camb. Philos. Soc.* **78**, 81–118. (doi:10.1017/S1464793102005985)
3. Hart NS. 2001 The visual ecology of avian photoreceptors. *Prog. Retin. Eye Res.* **20**, 675–703. (doi:10.1016/S1350-9462(01)00009-X)
4. Ödeen A, Håstad O. 2003 Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. *Mol. Biol. Evol.* **20**, 855–861. (doi:10.1093/molbev/msg108)
5. Maia R, Eliason CM, Bitton P, Doucet M, Shawkey MD. 2013 pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol. Evol*. **4**, 906–913. (doi:10.1111/2041-210X.1206)
6. Jaksic FM, Feisinger P. 1991 Bird Assemblages in Temperate Forests of North and South America: a Comparison of Diversity, Dynamics, Guild Structure and Resource Use. *Rev. Chil. Hist. Nat* **64**, 491–510.
7. Vergara PM, Armesto JJ. 2009 Responses of Chilean forest birds to anthropogenic habitat fragmentation across spatial scales*. Landsc. Ecol.* **24**, 25–38. (doi:10.1007/s10980-008-9275-y)