

**The Council of the Museum and Institute of
Zoology PAS in Warsaw**
Twarda 51/55
00-818 Warsaw

through:
The Council of Scientific Excellence
pl. Defilad 1
00-901 Warsaw
(Palace of Science and Culture, 24th floor,
room 2401)

Alfredo Attisano

.....
(name of applicant)

Museum and Institute of Zoology Polish Academy of Sciences

.....
(name of employer/academic institution unit)

Application

dated ...23/01/2026.....

re.: commencement of the procedure for the conferment of the post-doctoral degree of doctor habilitated in the field of **exact and natural sciences** in the following discipline:
Biological Sciences.

Scientific achievement which entitles the applicant to commence the procedure for the conferment of the post-doctoral degree of doctor habilitated:

the scientific achievement entitled **“Coevolutionary arms race and nestling phenotypic variation in a brood parasite – host system from New Caledonia”**, comprises a thematic cycle of five articles published in peer-reviewed international scientific journals. The total impact factor of the journals in which the articles were published is **15.079**, and the total points according to the ministerial list is **620**.

Pursuant to art. 221 para 10 of the Higher Education and Science Act dated 20 July 2018. I hereby kindly request that the habilitation commission pass a resolution on the conferment of the post-doctoral degree of doctor habilitated in **open** voting.

I was advised of the following: The President of the Scientific Council of Excellence with its registered office in Warsaw (pl. Defilad 1, 24th floor, 00-901 Warsaw) is the Administrator of personal data collected under the procedure for the conferment of the post-doctoral degree of doctor habilitated. Contact us via e-mail: kancelaria@rdn.gov.pl, phone 22 656 60 98 or personally at our office. Personal data shall be processed pursuant to art. 6 para 1 letter c) Regulation (EU) 2016/679 dated 27 April 2016 in connection with art. 220-221 and art. 232-240 of the Higher Education and Science Act dated 20 July 2018, for the purposes of the procedure for the conferment of the post-doctoral degree of doctor habilitated and in order to exercise the rights and obligations as well as the right to appeal in this procedure. For detailed information on processing personal data in the procedure see www.rdn.gov.pl/klauzula-informacyjna-rodo.html



.....
(Applicant's signature)

Appendices:

Appendix 1. Personal data of the applicant

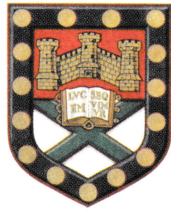
Appendix 2. Confirmation on obtaining the PhD title

Appendix 3. Summary of professional accomplishments

Appendix 4. List of scientific or artistic achievements

Appendix 5. Copies of 5 scientific publications constituting a thematic cycle

Appendix 6. Declarations of co-authors on their contribution



UNIVERSITY OF EXETER

We the undersigned hereby certify that

Alfredo Attisano

*having pursued an approved programme of study
and having fulfilled the requirements of the
Ordinances and Regulations of the University
was awarded the Degree of*

Doctor of Philosophy

by the Senate of the University on

4 March 2013



Hobella Benjamin

CHANCELLOR

Steve Smith
VICE-CHANCELLOR

Q. S. Pies

CHIEF OPERATING OFFICER

University Reference 590047993/1
Surname ATTISANO
Forenames ALFREDO

Date of Birth 10/Aug/1979
HESA Reference 0911190067725
Date of Entry 05/Oct/2009

Name of Qualification:
Field of Research:

Doctor of Philosophy
Life-History Variation And Evolved Response To Food Stress In
Oncopeltus Fasciatus (Hemiptera: Lygaeidae)

Language of Instruction:

Awarded on 4 March 2013
English

Programme Details (for further programme details please refer to the relevant programme specification at www.exeter.ac.uk/progspecs/).

2009/0 PHD Biological Sciences - C
Stage 1 Full Time

Attempt ECTS Credit Mark

Progress: Progress pass 30/Sep/2010

2010/1 PHD Biological Sciences - C
Stage 2 Full Time

Attempt ECTS Credit Mark

Progress: Progress pass 30/Sep/2011

2011/2 PHD Biological Sciences - C
Stage 3 Full Time

Attempt ECTS Credit Mark

Progress: Progress pass 11/Oct/2012

2012/3 PHD Biological Sciences - C
Stage 4 Awaiting awards (Prev FT) No registration

Attempt ECTS Credit Mark

Progress: Completed 04/Mar/2013

*=Exempt Module, Pathway or Award. C=Condoned

Date Transcript Issued: 5 March 2013

MARKS OR RESULTS MAY NOT BE RELEASED TO A THIRD PARTY WITHOUT THE STUDENT'S WRITTEN CONSENT



M Shoebri
Director of Academic Services
University of Exeter

For information on the award and the awarding institution, please refer to the reverse of this transcript.



Summary of professional accomplishments

1. Name

Alfredo Attisano

2. Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation.

2007 – Master’s Degree in Natural Sciences, Department of Zoology at Naples University “Federico II”, Italy. Title of master’s thesis: “The genetic tool for the conservation and management of wildfowl in Campania, Southern Italy”, supervisor: prof. Maria Filomena Caliendo, dr. Daniela Ripa.

2013 – Ph.D. in Biological Sciences, Centre for Ecology and Conservation, Faculty of Biosciences, University of Exeter, UK. Title of Ph.D. dissertation: “Life-history variation and evolved response to food stress in *Oncopeltus fasciatus* (Hemiptera:Lygaeidae)”, supervisors: prof. Patricia J. Moore, prof. Allen J. Moore, prof. Tom Tregenza.

3. Information on employment in research institutes or faculties/departments or school of arts.

09.2005 – 04.2007 - Research assistant. Laboratory of Population Genetics, Department of Zoology, University of Naples “Federico II”, Italy

09.2009 – 03.2013 - Ph.D. student. Centre for Ecology and Conservation, Faculty of Biosciences, University of Exeter, UK.

09.2010 – 05.2012 - Teaching assistant. Centre for Ecology and Conservation, Faculty of Biosciences, University of Exeter, UK.

03.2013 – 11.2014 - Postdoctoral research assistant. Behavioural Ecology Group, Department of Zoology, University of Cambridge, UK.

05.2015 – current - Assistant Professor (Adjunkt). Museum and Institute of Zoology, Polish Academy of Sciences.

4. Description of the achievements, set out in art. 219 paragraph 1 point 2 of the Act from 20 July 2018.

4.1 A series of five scientific articles published in 2018–2025, which constitute my achievement entitled “Coevolutionary arms race and nestling phenotypic variation in a brood parasite – host system from New Caledonia”.

Publications included in the thematic cycle of the scientific achievement:

1. **Attisano, A.,** Sato, N. J., Tanaka, K. D., Okahisa, Y., Kuehn, R., Gula, R., Ueda, K., & Theuerkauf, J. (2018). Visual discrimination of polymorphic nestlings in a cuckoo-host system. *Scientific Reports*, 8: 1–10. <https://doi.org/10.1038/s41598-018-28710-5> [IF₂₀₁₈ = 4.011; Q1; MNiSW points = 140]

I declare that my contribution to the above article consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. I am first author and corresponding author.

2. **Attisano, A.,** Hlebowicz, K., Gula, R., & Theuerkauf, J. (2021). Threat recognition and response in an avian brood-parasite host from New Caledonia. *Current Zoology*, 67: 255–262. <https://doi.org/10.1093/cz/zoaa061> [IF₂₀₂₁ = 2.734; Q1; MNiSW points = 100]

I declare that my contribution to the above article consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. I am first author and corresponding author.

3. **Attisano, A.,** Sato, N. J., Tanaka, K. D., Okahisa, Y., Ueda, K., Gula, R., & Theuerkauf, J. (2021). Discrimination and ejection of eggs and nestlings by the fan-tailed gerygone from New Caledonia. *Current Zoology*, 67: 653–663. <https://doi.org/10.1093/cz/zoab066> [IF₂₀₂₁ = 2.734; Q1; MNiSW points = 100]

I declare that my contribution to the above article consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. I am first author and corresponding author.

4. **Attisano, A.,** Gill, B.J., Anderson, M.G., Gula, R., Langmore, N.E., Okahisa, Y., Sato, N.J., Tanaka, K., Thorogood, R., Ueda, K., Theuerkauf, J. (2023). Polymorphism at the nestling stage and host-specific mimicry in an Australasian cuckoo-host arms race. *Journal of Animal Ecology* 93: 30-43, <https://doi.org/10.1111/1365-2656.13849> [IF₂₀₂₃ = 3.5; Q1; MNiSW points = 140]

I declare that my contribution to the above article included organising and managing data provided by all co-authors, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. In relation to the section from New Caledonia in the article, my contribution consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field and conducting the statistical analysis. I am first author and corresponding author.

5. **Attisano, A.,** Anderson M.G., Langmore N.E., Gula, R., Theuerkauf J. (2025). Begging call mimicry and formation of host-specific lineages in the shining bronze-cuckoo *Chalcites lucidus*. *Animal Behaviour* 221:123083, <https://doi.org/10.1016/j.anbehav.2025.123083> [IF₂₀₂₅ = 2.3; Q1; MNiSW points = 140]

I declare that my contribution to the above article consisted in formulating the main research hypothesis, organising and managing data provided by all co-authors, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. In relation to the section from New Caledonia in the article, my contribution consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field and conducting the statistical analysis. I am first author and corresponding author.

The total value of the Impact Factor (IF) of the journals in which the papers were published is 15.079, and the total number of MNiSW points is 620. The publications included in the list of achievements are the result of work financed by the grants 2012/05/E/NZ8/02694 and 2016/23/B/NZ8/03082 (publication 1, 3, 4), 2016/23/B/NZ8/03082 (publication 2) and 2022/45/B/NZ8/03740 (publication 5). I am the Primary Investigator in the last two grants. The project 2016/23/B/NZ8/03082 was successfully completed on 05.07.2021, whereas the project 2022/45/B/NZ8/03740 is ongoing and will end on the 07.01.2027. Two publications included in this achievement are the result of an international collaboration between ornithologists from Poland and Japan (publications 1 and 3) and two others result from an international collaboration of ornithologists from Poland, Japan, Australia and New Zealand (publications 4 and 5).

4.1.1 Introduction

Avian brood parasites lay eggs in nests of other birds, their hosts, which are left to care for the parasite offspring incurring in a high reproductive cost (Soler 2017). Hosts are under selection to develop defences against parasitism, which in turn promotes selection for improved

offences in brood parasites to evade host defences. These interactions may result in a coevolutionary arms race between hosts and brood parasites (Dawkins and Krebs 1979; Davies 2011).

Many hosts can recognise the eggs of brood parasites in their nest and much research has been devoted to the study of the factors underlying discrimination (Brooke and Davies 1988; Antonov et al. 2009; Spottiswoode and Stevens 2011; Caves et al. 2015; Hanley et al. 2021) and rejection of the parasite egg by the host (Marchetti 2000; Spottiswoode and Stevens 2010; Honza and Cherry 2017; Ruiz-Raya and Soler 2020; Samaš et al. 2021). It is also well established that the arms race at the egg stage can lead to variation in egg appearance in both the host and brood parasite (Takasu 2003; Spottiswoode and Stevens 2012; Medina et al. 2020; Yang et al. 2020). Therefore, a large number of studies conducted over the course of 40 years has given us a grounded understanding of the selective factors shaping interactions at the egg stage of the host breeding cycle (Davies 2015; Soler 2017).

By contrast, host defences against nestlings of brood parasites are rare (Soler 2009; Grim 2011). Direct evidences of host defences against parasite nestlings have been found in hosts from Oceania (Langmore et al. 2003; Sato et al. 2010, 2015; Tokue and Ueda 2010), whereas in other areas of the world hosts always accept the parasite nestlings (Grim 2017) or might reject the parasite only at the fledgling stage (Grim et al. 2003; De Mársico et al. 2012). As a consequence, we have a limited understanding of the selective factors driving interactions at the nestling stage of the arms race (Grim 2017).

In my achievement I focus on the co-evolutionary interactions occurring at the nestling stage of the arms race in a brood parasite – host system from the remote archipelago of New Caledonia. Here the fan-tailed gerygone (*Gerygone flavolateralis*) is the exclusive host of the shining bronze-cuckoo (*Chalcites lucidus*). The gerygone does not reject the parasite egg, however it rejects the parasite nestling by removing it from the nest usually within 24 hours from hatching. In this system, host discrimination of the parasite nestling promoted selection for visual mimicry of the host nestlings by the cuckoo to evade rejection (Sato et al. 2015). Furthermore, both the host and parasite have nestlings with two distinct skin phenotype, bright and dark, indicating further selection for phenotypic variation in nestlings' appearance in this system (Sato et al. 2015). The broods of the gerygone host can be monomorphic (either bright or dark) or mixed with the two nestling phenotypes coexisting together in the same nest. Despite phenotypic and within-brood variation, the gerygone host always rejects the cuckoo irrespective of the brood type, but never reject own nestlings by mistake. Nestling phenotypic variation is rare in birds (Kilner 2006) and no other system in the world has reached a level of

complexity in nestling appearance similar to what observed in New Caledonia. Therefore, the gerygone-cuckoo system from New Caledonian offers the unique opportunity to investigate parasite-host interactions at the nestling stage and understand the selective factors driving host discrimination of the parasite nestling and nestling phenotypic variation within a coevolutionary arms race.

My selected series of articles describes the modalities of cuckoo mimicry of the host nestlings (publication 1) and show how weak or absent defences against the parasite at earlier stages of the host breeding cycle might have promoted selection for refined defences at later stages of the arms race in this system (articles 2, 3). I also investigated which clues host parents use to discriminate a cuckoo nestling from their own (article 3). Finally, by looking at patterns of nestling phenotypic variation and mimicry across the distribution range of the shining bronze-cuckoo in the Pacific region, I show how the coevolutionary interactions with its hosts have promoted nestling diversification and contributed to the evolution of geographically and genetically separated cuckoo subspecies (articles 4, 5).

4.1.2 Results

1. **Attisano, A.,** Sato, N. J., Tanaka, K. D., Okahisa, Y., Kuehn, R., Gula, R., Ueda, K., & Theuerkauf, J. (2018). Visual discrimination of polymorphic nestlings in a cuckoo-host system. *Scientific Reports*, 8: 1–10.

The aim of this study was to understand how shining bronze-cuckoo nestlings mimic the appearance of fan-tailed gerygone nestlings. During the 2013-2014 field seasons (funded by the grant 2012/05/E/NZ8/02694) researchers from the Behavioural Ecology Unit at the Museum and Institute of Zoology PAS and the Behavioural Ecology Group at Rikkyo University Tokyo observed two distinct nestling phenotypes in the fan-tailed gerygone, a bright and a dark, but only a bright phenotype for the cuckoo nestlings. Analysis via reflectance spectra showed that the bright cuckoo nestlings matched more closely the bright host phenotype, thus suggesting mimicry of the bright host nestlings by the cuckoo (Sato et al. 2015). However, reflectance spectra do not take into account whole complex surfaces such as the entire nestlings' body, therefore the results of Sato et al., 2015 offered only a partial interpretation of the mimicry modalities of the host nestlings by the shining bronze-cuckoo nestlings.

In 2015 I joined the Behavioural Ecology Unit at the Museum and Institute of Zoology PAS as a postdoc to further investigate the modalities of host mimicry of the shining bronze-cuckoo nestlings. I used multispectral photography of nestlings, which allows to analyse visual

information from larger areas of the nestling's body and therefore achieve a more comprehensive and informative visual analysis compared to reflectance spectra. I used images collected by my colleagues and myself during 2013-2015 (supported by the grant 2012/05/E/NZ8/02694 in which I was a postdoc) and by myself in 2017 (supported by the grant 2016/23/B/NZ8/03082 in which I was the principal investigator). I analysed images of nestlings using models of avian vision to approximate how nestlings appear in the eyes of the gerygone host parents. I then compared the three nestling types (bright host, dark host and bright cuckoo) via the chromatic (colour) and achromatic (luminance) components of the three areas of the nestlings' body: dorsum (whole dorsal area including skin and natal down feathers), lateral flanges (fleshy lateral parts of the nestling's bill) and skin alone. I found that the two host nestlings' phenotypes appear more different from each other than to the cuckoo nestlings and that the bright cuckoo nestlings mimicked several chromatic and achromatic visual features of both host nestling morphs. Therefore, the bright cuckoo nestlings appear intermediate between the two host morphs rather than being a perfect match of the bright host alone. The results of this study thus suggests that bright cuckoo nestlings are under selection for intermediate mimicry, which would maximise the chances of acceptance of the bright cuckoo nestling by the host regardless of the host brood phenotype (bright, dark or mixed) they hatch in. At the same time, the relative frequencies of the host brood phenotypes at our sites might create a frequency dependent selection scenario in which the dark cuckoo nestlings are less adaptive than the bright phenotype and might be gradually disappearing from the local population, thus explaining the rarity of this phenotype at our field sites.

2. **Attisano, A.,** Hlebowicz, K., Gula, R., & Theuerkauf, J. (2021a). Threat recognition and response in an avian brood-parasite host from New Caledonia. *Current Zoology*, 67: 255–262

The aim of this study was to understand if fan-tailed gerygone adults recognise adult cuckoos around the nest and have adaptive defence responses to decrease parasitism risk.

Fan-tailed gerygone pairs could recognise shining bronze-cuckoo adults around the nest as a brood parasite threatening the survival of their eggs and thus present anti-parasitism defences at the frontline stage, i.e. before the cuckoo lay its egg in the host nest (Feeney et al. 2012). Specifically, we would expect that gerygonas attack cuckoos approaching active nests, that attacks are more frequent during incubation and that individuals exposed to brood parasites have higher parasitism and nestling rejection rates. However, the shining bronze-cuckoo also removes eggs or nestlings from ~13% of the active host nests without parasitising these, thus

acting as a nest predator (Attisano et al. 2020). Therefore, gerygone pairs could recognise the shining bronze-cuckoo as a small nest predator threatening brood or clutches but not their own life. We would then expect again that gerygones attack cuckoos approaching active nests, but also no difference in attack frequency between incubation and brooding and no difference in parasitism and nestling rejection rates between individuals mobbing the cuckoo and those who do not. If frontline defences are absent or ineffective in preventing parasitism then this could lead to increased selection for the evolution of improved defences at later stages of the host breeding cycle.

We placed models of 1) an adult shining bronze-cuckoo as a brood parasite and small nest predator, 2) a crow as a large nest predator and 3) a chaffinch as an unknown bird species at active nests of fan-tailed gerygone and quantified their behavioural response to the three models. Fan-tailed gerygone attacked only the cuckoo model, approached but not attacked the chaffinch model and always avoided the crow model. Nests from pairs that attacked the cuckoo model had similar parasitism rates as nests from pairs that did not attack the cuckoo model. Attack rates were similar between incubation and brooding stages. Finally, nestling rejection rates did not increase following exposure to the cuckoo model.

The results show that the gerygone host recognises adult cuckoos from other birds. However, their behaviour fits more the assumptions of a response to a small nest predator than to a brood parasite. Therefore, frontline defences in the fan-tailed gerygone are absent or ineffective in preventing parasitism, which allows selection for improved host defences at later stages of the arms race in this system.

3. **Attisano, A.,** Sato, N. J., Tanaka, K. D., Okahisa, Y., Ueda, K., Gula, R., & Theuerkauf, J. (2021b). Discrimination and ejection of eggs and nestlings by the fan-tailed gerygone from New Caledonia. *Current Zoology*, 67: 653–663.

The aim of this study was to understand if fan-tailed gerygone can discriminate and remove cuckoo eggs from the nest and which cues do fan-tailed gerygone parents use to recognise cuckoo nestlings from their own. The brown-olive eggs of the shining bronze-cuckoo are ~20% larger and appear very different from the whitish brown-spotted eggs of the fan-tailed gerygone. Despite this, we have never observed gerygones rejecting cuckoo eggs. The absence of egg rejection could thus result from the cuckoo eggs either 1) evading host detection due to their dark coloration (Langmore et al. 2009) or 2) being too large to be grasped by the small gerygone host (Marchetti 2000). I tested these hypotheses by introducing artificial eggs of different colours (cuckoo-like, host-like, blue) and sizes (50% smaller than gerygone

eggs, same size of real cuckoo and gerygone eggs) in active nests of fan-tailed gerygone during the incubation stage. I found that fan-tailed gerygone adults grasped and rejected artificial eggs regardless of their colour only if these were smaller than real eggs, but they never rejected artificial eggs matching the size of real cuckoo and gerygone eggs. Therefore, gerygones do discriminate brown-olive cuckoo eggs in their nest but these are too large to be grasped and rejected.

Additionally, I tested which cues do fan-tailed gerygone parents use to discriminate cuckoo nestlings from their own. I tested for recognition of nestlings via colour cues by introducing artificial nestlings mimicking the colour of real gerygone nestlings (bright, dark). I tested for recognition of the nestlings via natal down and timing of hatching by introducing cross-fostered nestlings that 1) had their natal down feather present or removed and 2) were introduced before or after hatching of the first host chick in the brood. Finally, I recorded begging calls of gerygone and cuckoo nestlings while being fed by fan-tailed gerygone parents in active nests. I found that artificial nestlings were rejected at lower rates than real nestlings and that their colour did not influence rejection rates. Rejection rates of cross-fostered nestlings were higher when they lacked their natal down and were introduced in the nest before hatching of the brood in the experimental nest. Begging calls differed significantly between parasite and host nestlings throughout the nestling period.

The results show that defences at the egg stage of the fan-tailed gerygone are ineffective in preventing parasitism, similar to what I found with the defences at the frontline stage (Attisano et al., 2021a). The results of these two studies suggests increased selection pressure for the evolution of nestling defences in the fan-tailed gerygone host as the only effective method to significantly decrease the negative impact of brood parasitism. The selection for defences at the nestling stage is favoured by the fact that nestlings provide multiple cues that can facilitate discrimination by host parents as indeed my results show that gerygone parents can discriminate nestlings via their natal down, hatching order, begging calls and visual appearance.

4. **Attisano, A.,** Gill, B.J., Anderson, M.G., Gula, R., Langmore, N.E., Okahisa, Y., Sato, N.J., Tanaka, K., Thorogood, R., Ueda, K., Theuerkauf, J. (2023). Polymorphism at the nestling stage and host-specific mimicry in an Australasian cuckoo-host arms race. *Journal of Animal Ecology* 93: 30-43

The aim of this study was to describe how the arms races between the shining bronze-cuckoo and its hosts from Australia, New Caledonia and New Zealand has resulted in

phenotypic diversification at the nestling stage and contributed to the separation in different cuckoo subspecies. In each region the cuckoo parasitises hosts in the family Acanthizidae with similar breeding and nesting habits. Some hosts have only moderately effective defences at the frontline and egg stages, whereas other hosts reject the cuckoo nestlings at hatching. The nestlings of the three cuckoo subspecies appear visually distinct from each other and resemble more closely the phenotype of the nestlings of the local host in each region. We also found phenotypic variation in nestlings of cuckoos and hosts in some regions but not in others. The patterns of host defences and nestling phenotypic variation across regions suggests different evolutionary stages of the arms race in which either the cuckoo or the host is in the lead. Hosts having moderately defences at the frontline and egg stages do not appear to have defences at the nestling stage and viceversa. In areas where the parasite nestling is a better visual match of the host nestlings rejection of the cuckoo nestling does not occur, or is too rare to be detected during field studies.

This study is the first to show how interactions at the nestling stage between a cuckoo and its hosts over a wide geographic scale can lead to host specialisation and nestling phenotypic variation. It also highlights the importance of brood parasitism in promoting phenotypic diversification and contributing to the speciation process via the formation of separate subspecies.

5. **Attisano, A.,** Anderson M.G., Langmore N.E., Gula, R., Theuerkauf J. (2025) Begging call mimicry and formation of host-specific lineages in the shining bronze-cuckoo, *Chalcites lucidus*. *Animal Behaviour* 221:123083

The aim of this study, together with my previous study on visual mimicry (Attisano et al., 2023), was to find support for the hypothesis that nestlings of three shining bronze-cuckoo subspecies have evolved multimodal mimicry of their respective host nestlings. Brood parasites can diversify into host-specific populations mimicking visual features of the host's offspring, such as the appearance of eggs and nestlings (Medina et al. 2020). However, evidence of mimicry of begging calls and acoustic host-specialisation in brood parasites are still rare (Jamie and Kilner 2017). This study follows similar premises of study 4 in this list of achievements, i.e. detect mimicry of the local host by each shining bronze-cuckoo subspecies and therefore evidence of host specialisation. While study 4 was aimed at detecting and describing visual mimicry of the shining bronze-cuckoo subspecies, this study investigates the possibility of acoustic mimicry between each cuckoo subspecies and the respective local host across their distribution range in the Pacific region.

I found that nestlings of shining bronze-cuckoo subspecies from Australia, New Caledonia and New Zealand produce begging calls matching those of their respective main host in each region, therefore showing acoustic host-specialisation. The matching is more accurate in areas where the host does not reject cuckoo nestlings. Furthermore, the begging calls of each cuckoo subspecies are more similar to the begging calls of their respective hosts than they are to each other. Therefore, coevolutionary interactions, rather than phylogenetic relationships, drive selection for acoustic diversification on the structure of cuckoo begging calls.

The studies 4 and 5 in this list of achievements show that the coevolutionary interactions in these host-cuckoo systems have promoted refined visual and auditory mimicry of the host by the shining bronze-cuckoo nestlings. These cuckoo-host systems are excellent models to investigate coevolutionary mechanisms and understand their contribution in phenotypic diversification and formation of geographically isolated evolutionary units across the south-Pacific region.

4.1.3 Summary

The results presented in my list of achievements show that:

- bright cuckoo nestlings of the shining bronze-cuckoo from New Caledonia mimic both nestling morphs of its fan-tailed gerygone host. This suggests selection for an intermediate morph, which increases the likelihood of acceptance of the cuckoo nestling by the host parents regardless of the phenotype of the host brood (Attisano et al. 2018)
- ineffective defences at the frontline and egg stages of the host breeding cycle have promoted selection for defences against the parasite nestling in the fan-tailed gerygone. This host cannot avoid parasitism at the laying stages (Attisano et al. 2021a) and cannot reject the cuckoo eggs due to morphological constraints (Attisano et al. 2021b). Therefore, the only effective defence against parasitism for this host is to reject the parasite nestling. Parasite nestlings offer multiple cues to host parents compared to the parasite adults and eggs, thus facilitating nestling discrimination and the evolution of a nestling rejection strategy (Attisano et al. 2021b)
- the shining bronze-cuckoo present host specialisation across its distribution range in the south Pacific region, with parasite nestlings mimicking visual and acoustic features of the nestlings of the respective local hosts in each region (Attisano et al. 2023, 2025). Coevolutionary interactions promoted phenotypic diversification in these parasite-host systems and contributed to the formation of isolated shining bronze-cuckoo subspecies.

4.1.4 References

- Antonov A, Stokke BG, Moksnes A, Røskaft E, 2009. Evidence for egg discrimination preceding failed rejection attempts in a small cuckoo host. *Biol Lett* 5:169–171.
- Attisano A, Anderson MG, Langmore NE, Gula R, Theuerkauf J, 2025. Begging call mimicry and formation of host-specific lineages in the shining bronze-cuckoo, *Chalcites lucidus*. *Anim Behav* 221:123083.
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4.2 Other selected scientific achievement

My other selected achievement is a series of three articles published in the period 2013–2015, in which I developed a techniques to study insect flight in laboratory settings and used it to

analyse the physiological drivers of migratory behaviour in the large milkweed bug and parental effects on wing morphology and flight performance in the burying beetle. These articles are among the most cited in my list of scientific achievements. All papers have been published after obtaining my PhD.

1. **Attisano, A.,** Tregenza, T., Moore, A. J., & Moore, P. J. (2013). Oosorption and migratory strategy of the milkweed bug, *Oncopeltus fasciatus*. *Animal Behaviour*, 86: 651–657. <https://doi.org/10.1016/j.anbehav.2013.07.013> [IF₂₀₁₃ = 3.068; Q1; MNiSW points = 140]

I declare that my contribution to the above article consisted in formulating the main hypothesis, designing and implementing the methodology, collecting data in the field and in the laboratory, performing statistical analysis, preparing figures and tables, writing the first version of the manuscript. I am the first and corresponding author.

In this study I used the technique I developed to measure insect flight in laboratory settings (number 3 in this list of achievements) to investigate the interaction between reproductive physiology and migratory behaviour in the large milkweed bug *Oncopeltus fasciatus*. This insect has a partial migratory strategy, i.e. within a population some individuals migrate whereas others do not. I found that physiological adjustments of the reproductive system in response to food shortages correlate with differences in the migratory behaviour, therefore linking reproductive physiology with behavioural adaptations to cope with unfavourable environmental conditions.

2. **Attisano, A.,** & Kilner, R. M. (2015). Parental effects and flight behaviour in the burying beetle, *Nicrophorus vespilloides*. *Animal Behaviour*, 108: 91–100. <https://doi.org/10.1016/j.anbehav.2015.07.020> [IF₂₀₁₅ = 3.169; Q1; MNiSW points = 140]

I declare that my contribution to the above article consisted in formulating the main hypothesis, designing and implementing the methodology, collecting data in the field and in the laboratory, performing statistical analysis, preparing figures and tables, writing the first version of the manuscript. I am the first and corresponding author.

In this study I used a modified version of the flight mills I develop (article 3) to study how parental care influences offspring morphology and behavioural performances in the burying beetle *Nicrophorus vespilloides*. I found that different regimes of parental care (full care, no care) have a significant effect on the wing morphology of offspring in adulthood. Differences in wing morphology are associated with variation in flight behaviour and performances and ultimately influence the ability of individuals in competing for resources in adulthood.

3. **Attisano, A.,** Murphy, J. T., Vickers, A., & Moore, P. J. (2015). A Simple flight mill for the study of tethered flight in insects. *JoVE (Journal of Visualized Experiments)*, 106: e53377. <https://doi.org/10.3791/53377> [IF₂₀₁₅ = 1.113; Q2; MNiSW points = 70]

I declare that my contribution to the above article consisted in designing and building the flight mills, writing and testing the code, collecting data, performing statistical analysis, preparing figures and tables, writing the first version of the manuscript. I am the first author.

In this article I describe the flight mills I developed during my PhD to analyse insect flight in laboratory settings. The article provides a step-by-step guide to build a flight mill based on my design. The article also provides the full code, which I wrote in Python, to analyse individual flight data and extract statistics on flight performance.

5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions.

5.1 Research activity

I conducted my scientific activity at research institutions in Poland and abroad: University of Exeter, University of Cambridge, University of Naples and Museum and Institute of Zoology PAS. I also collaborated with scientists from a large number of research institutions around the world such as the Australian National University (Australia), University of Vienna (Austria), University of Helsinki (Finland), Technical University of Munich (Germany), Max Plank Institute for Ornithology (Germany), University of Giessen (Germany), University of Bielefeld (Germany), IRD Noumea (New Caledonia), Auckland Museum (New Zealand), Massey University (New Zealand), Donana Biological Station (Spain), University of Stockholm (Sweden), University of Zurich (Switzerland), University of Lausanne (Switzerland), Rikkyo University (Tokyo), University of Georgia (USA), University of Exeter (UK), University of Cambridge (UK) and others.

I authored 22 research articles in specialists and multidisciplinary international journals listed in Web of Science. I am the first author in 11 publications and corresponding author in 8. The full list of my research articles is included in appendix 4, section II.8. My research has focused on the behavioural and evolutionary ecology of insects and birds, in particular on physiological and behavioural responses to challenging environments, parental effects and evolutionary change, brood parasitism, breeding phenology and conservation

Before obtaining my PhD

In 2006-2007 I worked as undergraduate research assistant and was involved in several wildlife monitoring projects at the Laboratory of Population Genetics at the Department of Zoology of the University of Naples. During this period I gained essential field and laboratory skills that have helped me greatly through my academic career thus far.

In 2009 I was awarded a PhD scholarship at the Centre for Ecology and Conservation of the University of Exeter funded by the European Social Fund (ESF). In my PhD dissertation I focused on the reproductive physiology of the large milkweed bug *Oncopeltus fasciatus* as a mechanism to cope with challenging environmental conditions. During my PhD I published two articles focusing on reproductive physiology (Moore and Attisano, 2011) and food quality as a mediator of the reproduction-longevity trade-off (Attisano et al., 2012). A third article focused on the link between reproductive physiology and partial migration behaviour in insects as an adaptive mechanism to cope with environmental uncertainty (Attisano et al., 2013) and was published after completing my PhD. During my PhD I designed and built a flight mill for the study of tethered flight in insects under laboratory conditions and described the methodology in a paper I published after obtaining my PhD (Attisano et al. 2015).

After obtaining my PhD

In 2013-2014 I held a postdoctoral position in the Behavioural Ecology Group at the Department of Zoology, University of Cambridge working with Prof Rebecca Kilner. I worked on parental care as a mediator of evolutionary change using the burying beetle *Nicrophorus vespilloides* as a model species. I used the flight mills I designed during my PhD to investigate how parental effects influence wing development and flight behaviour in offspring (Attisano and Kilner, 2015). I was also involved in studies investigating how different regimes of parental care during development induce changes in parental behaviour of the offspring in adulthood (Kilner et al., 2015) and in the role of interspecific interactions between beetles and their phoretic mites in inducing plastic responses to environmental uncertainty (De Gasperin et al., 2019).

In 2015 I joined the Behavioural Ecology Unit of the Museum and Institute of Zoology PAS as a postdoctoral researcher to work on the nestling colour polymorphism and coevolutionary interactions between the brood parasitic shining bronze-cuckoo *Chalcites lucidus* and its host fan-tailed gerygone *Gerygone flavolateralis* from New Caledonia. The position was part of an international collaboration of researchers from the Museum and Institute of Zoology (Poland), Rikkyo University (Japan) and Technische Universität München (Germany). The results of this work were published in two articles in which I looked at the

modalities of visual mimicry by the cuckoo nestling of the gerygone host (Attisano et al., 2018, included in the thematic cycle of my main scientific achievement) and the influence of extra-pair paternity in determining phenotypic variation in fan-tailed gerygone nestlings (Bojarska et al., 2018).

In 2017 – 2021 I was the Principal Investigator (PI) in a project funded by the National Science Center Poland (total funding 842 200 PLN). The aim of the project was to investigate the coevolutionary interactions and mechanisms of discrimination of the shining bronze-cuckoo nestlings by the fan-tailed gerygone host from New Caledonia. One aspect of this research focused on understanding aspects of the breeding biology of the fan-tailed gerygone and quantifying nest survival rates in relation to parasitism and predation pressures (Attisano et al., 2019, 2020). The other aspect focused on investigating the nest defence strategies of the fan-tailed gerygone host against brood parasites and nest predators and the mechanisms of discrimination of the eggs and nestlings of the brood parasite (Attisano et al., 2021a, 2021b, both papers are included in the thematic cycle of my main scientific achievement). I also lead an analysis using three long-term research studies spanning 40 years of field research from Australia, New Caledonia and New Zealand to elucidate the selective factors driving coevolutionary interactions and phenotypic diversification between three subspecies of the shining bronze-cuckoo and its hosts in Oceania (Attisano et al., 2023, included in the thematic cycle of the main scientific achievement).

In 2019 I was a founding member of the Gondwanan Ornithology Working Group of the International Ornithologists Union, which aim is to gather ornithologists working in the southern hemisphere and to foster collaboration and knowledge exchange. We published an article in *Trends in Ecology and Evolution* in which we denounced the bias in research efforts in behavioural, ecological and evolutionary biology between the two hemispheres and proposed ways to overcome such knowledge gap (Theuerkauf et al., 2022).

Since 2023 I am the Principal Investigator in a project funded by the National Science Center Poland (total funding 2 058 660 PLN). The aim of the project is to further investigate the coevolutionary interactions at the nestling stage between the shining bronze-cuckoo and fan-tailed gerygone in New Caledonia and understand the selective factors determining nestling phenotypic variation in these species. The project is ongoing and will end in January 2027. The first results from this project resulted in a study that looked at how mimicry of the host begging calls by the shining bronze-cuckoo nestlings can drive specialisation in this brood parasite and can lead to the formation of separate subspecies (Attisano et al., 2025, included in the thematic cycle of the main scientific achievement).

During 2024-2025 I was involved in studies aimed at developing methodologies to estimate populations size of elusive rainforest species (Theuerkauf et al., 2025a) and guide conservation planning of endangered birds in New Caledonia (Theuerkauf et al., 2025b). I have also collaborated with ornithologists from around the world to investigate worldwide patterns of bird vocalisations, in particular the structure of alarm calls in response to brood parasites and nest predators (Feeney et al., 2025) and methodologies for accurate recognition of avian species worldwide based on their vocalisations (Perez-Granados et al., 2025a, 2025b; Funosas et al. 2026).

5.2 Reviews for scientific journals

I have reviewed 66 manuscripts for international scientific journals including *Animal Behaviour* (9), *Animals* (26), *Avian Research* (2), *Behavioural Ecology* (1), *Behavioural Ecology and Sociobiology* (3), *Behavioural Processes* (1), *Biological Journal of the Linnean Society* (1), *Biology* (2), *Current Zoology* (1), *Diversity* (1), *Ecology and Evolution* (2), *Ecological Informatics* (7), *Ethology Ecology and Evolution* (1), *European Zoological Journal* (1), *Frontiers in Zoology* (1), *Forests* (3), *Integrative Zoology* (1), *Journal of Animal Ecology* (1), *Philosophical Transactions of the Royal Society B* (1), *Polish Journal of Ecology* (1).

5.3 Research projects

After obtaining my PhD, I participated in 5 research projects: 2 as PI, 2 as post-doc and 1 as collaborator.

- 1) Project funded by European Research Council (ERC). Project title: “The origin of the fittest: canalization, plasticity and selection as a consequence of provisioning during development”. Dates: 05.03.2013 – 30.11.2014. Role: postdoctoral research assistant.
- 2) Project funded by National Science Center, Poland, Sonata Bis 1. Project title: “Does an epigenetic mechanism escalate the co-evolutionary arms race in avian-host parasite systems?”. Dates: 05.05.2015 – 25.07.2017. Role: postdoctoral research associate.
- 3) Project funded by National Science Center, Poland, OPUS 12. Project title: “Nestlings’ phenotypic polymorphism and co-evolutionary arms race in a host-parasite system in New Caledonia”. Dates: 06.07.2017 – 05.07.2021, Role: Principal Investigator.
- 4) Project funded by National Science Center, Poland, OPUS 15. Project title: “Mate choice and family formation in a cooperatively breeding bird”. Dates: 09.01.2019 – 08.01.2024. Role: collaborator.

- 5) Project funded by National Science Center, Poland, OPUS 23. Project title: “The role of early environment and physiology on phenotypic variation of nestlings in an avian coevolutionary arms race”. Dates: 27.01.2023 – 26.01.2027. Role: Principal Investigator.

5.4 Scientific conferences

I have attended several scientific conferences in which I gave oral and poster presentations, either as a presenting author or as a co-author. The full list of attended conferences is given in Appendix 4, section II.2

5.5 Scholarships

- 1) PhD scholarship at the University of Exeter funded by European Social Fund “Geographic variation and evolved response to food stress”. 01.10.2009 – 30.09.2012

6. Presentation of teaching and organizational achievements as well as achievements in popularization of science and art

6.1 Teaching achievements

Before obtaining my PhD

During my PhD studies, as a Teaching Assistant I provided assistance to lectures, mentoring, supervision and pastoral care of undergraduate students for the courses of Evolutionary Ecology, Development and Diversity, Ecology and Conservation, Trends in Ecology and Evolution, Analysis of Biological Data. In 2010 I attended the course of Learning and Teaching in Higher Education and gained the certificate of LTHE (Learning and Teaching in Higher Education) phase 1. I also supervised three undergraduate students during the completion of their final year research project.

After obtaining my PhD

In 2013 – 2014, while employed as a postdoc at the Department of Zoology of the University of Cambridge, I supervised two undergraduate students for the completion of their final year project.

In 2017 – 2018 I acted as field research supervisor for a master student from the University of Bremen, Germany. The title of the master thesis was “Native birds but not invasive rodents are the main predators of the fan-tailed gerygone (*Gerygone flavolateralis*) in New Caledonia”.

Since 2022 I am coordinating and delivering the Behavioural Ecology course at the BioPlanet Doctoral School.

In 2023 – 2024 I supervised a master student from the University of Göttingen, Germany. The title of the master thesis was “Home range and territorial behaviour of fan-tailed gerygone (*Gerygone flavolateralis*) from New Caledonia”

Since 2023 I am co-supervisor of a PhD student from the Museum and Institute of Zoology, PAS. The title of the PhD project is “Ecological and physiological effects on nestlings’ phenotypic variation”.

In addition, I have taught classes at high school students. In 2009, while employed as Research Assistant by MATER Soc.Cons. in Naples, Italy, I organised and delivered courses in Land Use and Management, Recycling and Sustainability and Environmental Risk to high school students. During 2018 – 2022, I tutored high school students and delivered modules on Scientific Writing and Skills for Scientific Communication within a scholarship program funded by the Adamed Group, Poland. During 2022 – 2023, I delivered a course in Data Analysis and Programming in R to high school students from Akademeia High School, Poland.

6.2 Popularization of science

6.2.1 Lectures and short oral presentations

I have been invited to give seminars and short presentations 6 times in Poland and Great Britain.

1. Centre for Ecology and Conservation, University of Exeter. PhD seminar. Title: “Reproductive physiology and evolution of a partial migratory strategy”. 26.04.2011
2. Department of Zoology, University of Cambridge. Internal seminar. Title: “Parental effects and flight behaviour in the burying beetle”. 20.10.2013
3. Museum and Institute of Zoology, PAS. Seminar. Title: “The coevolutionary arms race in an avian host-parasite system from New Caledonia”. 17.03.2016
4. Day of the Italian Research in Poland, Italian Embassy, Warsaw. Short seminar. Title: “Behavioural ecology of birds from New Caledonia”. 22.06.2022
5. Museum and Institute of Zoology, PAS. Short internal seminar. Title: “Nestling phenotypic variation in a brood parasite-host system from New Caledonia”. 07.02.2023
6. Adam Mickiewicz University, Poznan. Seminar. Title: “Coevolution and nestling phenotypic variation in an avian brood parasite-host system from New Caledonia”. 19.03.2025

6.2.2 Media interviews

In 2018 I collaborated as scientific consultant for the production of a wildlife documentary on the behavioural ecology of birds from New Caledonia for NHK (Nippon Hōsō Kyōkai - Japan’s

main national TV channel). Part of the one-hour-long documentary focused on my research on the fan-tailed gerygone and shining bronze-cuckoo and featured an interview and outlook on my field research. The documentary was featured within the “*Wildlife*” documentary cycle, which is among the TV programmes with the largest audience in Japan.

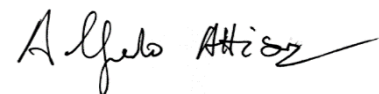
My research has been covered by online magazines such as *Gazeta Wiborcza* (2022), *Science in Poland* (2025), *Swiat Nauki* (2017).

7. Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important

I raised my professional qualifications by attending workshops and courses for professional development:

1. PoLASA certificate for working with laboratory animals, 2022
2. Machine learning. Stanford University Online course, 2021
3. Signal extraction, cluster analysis and acoustic indices in Kaleidoscope Pro. Wildlife Acoustics Training, Online course. 2021
4. R programming. John Hopkins University, Online course, 2015
5. R-objected programming and package development. University of Cambridge Bioinformatics Training. 2014
6. Programming with Python 3. University of Cambridge Training, 2014
7. Open source tools for GIS analysis and data processing. University of Exeter, 2012
8. Learning and Teaching in Higher Education, LTHE Phase 1. University of Exeter, 2010

(applicant signature)

A handwritten signature in black ink, appearing to read "Alfio Attis", with a long horizontal flourish extending to the right.

List of scientific or artistic achievements which present a major contribution to the development of a specific discipline

I. LIST OF SCIENTIFIC OR ARTISTIC ACHIEVEMENTS, REFERRED TO IN ARTICLE 219, SECTION 1. POINT 2 OF THE ACT

2. A cycle of thematically related scientific articles, in accordance with Article 219, Section 1. Point 2b of the Act

All articles have been published after obtaining my PhD.

1. **Attisano, A.,** Sato, N. J., Tanaka, K. D., Okahisa, Y., Kuehn, R., Gula, R., Ueda, K., & Theuerkauf, J. (2018). Visual discrimination of polymorphic nestlings in a cuckoo-host system. *Scientific Reports*, 8: 1–10. <https://doi.org/10.1038/s41598-018-28710-5> [IF₂₀₁₈ = 4.011; Q1; MNiSW points = 140]

I declare that my contribution to the above article consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. I am first author and corresponding author.

2. **Attisano, A.,** Hlebowicz, K., Gula, R., & Theuerkauf, J. (2021). Threat recognition and response in an avian brood-parasite host from New Caledonia. *Current Zoology*, 67: 255–262. <https://doi.org/10.1093/cz/zoaa061> [IF₂₀₂₁ = 2.734; Q1; MNiSW points = 100]

I declare that my contribution to the above article consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. I am first author and corresponding author.

3. **Attisano, A.,** Sato, N. J., Tanaka, K. D., Okahisa, Y., Ueda, K., Gula, R., & Theuerkauf, J. (2021). Discrimination and ejection of eggs and nestlings by the fan-tailed gerygone from New Caledonia. *Current Zoology*, 67: 653–663. <https://doi.org/10.1093/cz/zoab066> [IF₂₀₂₁ = 2.734; Q1; MNiSW points = 100]

I declare that my contribution to the above article consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. I am first author and corresponding author.

4. **Attisano, A.,** Gill, B.J., Anderson, M.G., Gula, R., Langmore, N.E., Okahisa, Y., Sato, N.J., Tanaka, K., Thorogood, R., Ueda, K., Theuerkauf, J. (2023). Polymorphism at the nestling stage and host-specific mimicry in an Australasian cuckoo-host arms race. *Journal*

of *Animal Ecology*, 93: 30-43, <https://doi.org/10.1111/1365-2656.13849> [IF₂₀₂₃ = 3.5; Q1; MNiSW points = 140]

I declare that my contribution to the above article included organising and managing data provided by all co-authors, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. In relation to the section from New Caledonia in the article, my contribution consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field and conducting the statistical analysis. I am first author and corresponding author.

5. **Attisano, A.**, Anderson M.G., Langmore N.E., Gula, R., Theuerkauf J. (2025). Begging call mimicry and formation of host-specific lineages in the shining bronze-cuckoo *Chalcites lucidus*. *Animal Behaviour*, 221:123083, <https://doi.org/10.1016/j.anbehav.2025.123083> [IF₂₀₂₅ = 2.3; Q1; MNiSW points = 140]

I declare that my contribution to the above article consisted in formulating the main research hypothesis, organising and managing data provided by all co-authors, conducting statistical analysis, preparing figures and tables and writing the first version of the manuscript. In relation to the section from New Caledonia in the article, my contribution consisted in formulating the main research hypothesis, obtaining funding, planning methodology, collecting data in the field and conducting the statistical analysis. I am first author and corresponding author.

4. Scientific or artistic achievements other than those listed in point I.2

The articles have been published after obtaining my PhD.

- 1) **Attisano, A.**, Tregenza, T., Moore, A. J., & Moore, P. J. (2013). Oosorption and migratory strategy of the milkweed bug, *Oncopeltus fasciatus*. *Animal Behaviour*, 86: 651–657. <https://doi.org/10.1016/j.anbehav.2013.07.013> [IF₂₀₁₃ = 3.068; Q1; MNiSW points = 140]

I declare that my contribution to the above article consisted in formulating the main hypothesis, designing and implementing the methodology, collecting data in the field and in the laboratory, performing statistical analysis, preparing figures and tables, writing the first version of the manuscript. I am the first and corresponding author.

- 2) **Attisano, A.**, & Kilner, R. M. (2015). Parental effects and flight behaviour in the burying beetle, *Nicrophorus vespilloides*. *Animal Behaviour*, 108: 91–100. <https://doi.org/10.1016/j.anbehav.2015.07.020> [IF₂₀₁₅ = 3.169; Q1; MNiSW points = 140]

I declare that my contribution to the above article consisted in formulating the main hypothesis, designing and implementing the methodology, collecting data in the field and in the laboratory, performing statistical analysis, preparing figures and tables, writing the first version of the manuscript. I am the first and corresponding author.

- 3) **Attisano, A.**, Murphy, J. T., Vickers, A., & Moore, P. J. (2015). A Simple flight mill for the study of tethered flight in insects. *JoVE (Journal of Visualized Experiments)*, 106: e53377. <https://doi.org/10.3791/53377> [IF₂₀₁₅ = 1.113; Q2; MNiSW points = 70]

I declare that my contribution to the above article consisted in designing and building the flight mills, writing and testing the code, collecting data, performing statistical analysis, preparing figures and tables, writing the first version of the manuscript. I am the first author.

II. LIST OF SCIENTIFIC OR ARTISTIC ACTIVITY

2. List of speeches at national or international scientific or artistic conferences, with specification of invited lectures and plenary lectures.

** presenting author*

Before obtaining my PhD

- 1) ***Attisano. A.**, Tregenza T., Moore A.J., Moore P.J. *Reproductive physiology and evolution of a partial migratory system* (poster). Meeting of the International Society for Behavioural Ecology, Lund, 12-17 August 2012

After obtaining my PhD

- 1) Theuerkauf J., Gula R., Sato N.J., Tanaka K.D, Okahisa Y., Kuehn R., Gazda M.A., **Attisano A.** *Escalation of the co-evolutionary arms race in an avian host-parasite system in New Caledonia* (oral presentation). Polish Evolutionary Conference, Poznań, 24-26 September 2015
- 2) Gazda M. A., Kuehn R., Sato N. J., Tanaka K. D., Ueda K., Okahisa Y., **Attisano A.**, Gula R., Theuerkauf J. *Mating system of the fan-tailed gerygone (*Gerygone flavolateralis*) and its role in a co-evolutionary host-cuckoo arms race* (poster). Polish Evolutionary Conference, Poznań, 24-26 September 2015
- 3) ***Attisano A.**, Sato N. J., Tanaka K. D., Okahisa Y., Kuehn R., Gula R., Ueda K., Theuerkauf J. *Visual discrimination of parasitic nestlings in a cuckoo-host polymorphic*

system (oral presentation). Polish Evolutionary Conference, Białystok, 25-27 September 2016

- 4) De Gasperin O., Duarte A., English, S., **Attisano A.**, Kilner R. *The early life environment pre-determines individual plasticity in a life history strategy* (poster). Meeting of the European Society for Evolutionary Biology, Groningen, 20-25 August 2017
- 5) ***Attisano A.**, Sato N. J., Tanaka K. D., Okahisa Y., Kuehn R., Gula R., Ueda K., Theuerkauf J. *Co-evolutionary arms race and visual discrimination of polymorphic nestlings in a cuckoo-host system of New Caledonia* (oral presentation). International Ornithological Congress, Vancouver, 19-26 August 2018
- 6) Theuerkauf J., **Attisano A.**, Gula R. *Cooperative breeding, clannish organisation, coevolutionary arms race, and other unique adaptations of birds in New Caledonia* (oral presentation). International Ornithological Congress, Vancouver, 19-26 August 2018
- 7) Walesiak M., Theuerkauf J., **Attisano A.**, Gula R. *Biogeographical analysis of the cuckoo-host relationships in New Caledonia* (poster). Polish Evolutionary Conference, Warsaw, 26-28 September 2018
- 8) Gula R., Kuehn R., Zagalska-Neubauer M., **Attisano A.**, Theuerkauf J. *Sex ratios and cooperative polyandry in the endangered Kagu of New Caledonia* (oral presentation). International Ornithological Congress, Virtual congress, 15-19 August 2022
- 9) ***Attisano A.**, Gula R., Theuerkauf J. *Do early environment or physiology determine nestling phenotypic variation?* (oral presentation). Meeting of the International Society for Behavioural Ecology, Melbourne, 29 September – 4 October 2024
- 10) **Attisano A.**, Anderson M.G., Langmore N.E., Gula R., Theuerkauf J. *Begging call mimicry and formation of host-specific lineages in the shining bronze-cuckoo* (oral presentation). New Zealand Bird Conference 2025, Auckland, 31 May – 2 June 2025

3. List of participation in organizational and scientific committees of national or international conferences, with specifying the function performed.

Founding member - Gondwanan Ornithology Working Group, International Ornithologists Union, 2019

4. List of participation in works of research teams implementing projects financed by way of national or international competitions, with division into completed projects and

projects in the course of performance and with taking into account the information on the function performed within the framework of works of the teams.

Before obtaining my PhD

1) Dates: 01.10.2009 – 30.09.2012

Role: PhD student

Funding: European Social Fund (ESF)

Project title: Life-history variation and evolved response to food stress in *Oncopeltus fasciatus* (Hemiptera:Lygaeidae)

After obtaining my PhD

Projects completed:

1) Dates: 05.03.2013 – 30.11.2014

Role: postdoctoral research assistant

Funding: European Research Council (ERC)

Project title: The origin of the fittest: canalization, plasticity and selection as a consequence of provisioning during development

2) Dates: 05.05.2015 – 25.07.2017

Role: postdoctoral research associate

Funding: Sonata Bis 1, National Science Center (NCN)

Project title: Does an epigenetic mechanism escalate the co-evolutionary arms race in avian-host parasite systems?

3) Dates: 06.07.2017 – 05.07.2021

Role: Pincipal Investigator

Funding: Opus 12, National Science Center (NCN)

Project title: Nestlings' phenotypic polymorphism and co-evolutionary arms race in a host-parasite system in New Caledonia

4) Dates: 09.01.2019 – 08.01.2024

Role: collaborator

Funding: Opus 15, National Science Center (NCN)

Project title: Mate choice and family formation in a cooperatively breeding bird

Project in progress:

1) Dates: 27.01.2023 – 26.01.2027

Role: Primary Investigator

Funding: Opus 23, National Science Center (NCN)

Project title: The role of early environment and physiology on phenotypic variation of nestlings in an avian coevolutionary arms race

5. List of membership in international or national organizations and scientific societies, together with information on the functions performed.

European Society for Evolutionary Biology – ordinary member since 2013

International Ornithological Union – ordinary member since 2018

International Society Behavioural Ecology – ordinary member since 2012

European Ornithologists' Union – ordinary member since 2017

8. List of peer-reviewed scientific or artistic works, particularly those published in international journals.

Before obtaining my PhD

1. Moore, P. J., & Attisano, A. (2011). Oosorption in response to poor food: complexity in the trade-off between reproduction and survival. *Ecology and Evolution*, 1: 37–45 <https://doi.org/10.1002/ece3.4> [IF₂₀₁₁ = 1.2; Q3; MNiSW points = 100]
2. Attisano, A., Moore, A. J., & Moore, P. J. (2012). Reproduction-longevity trade-offs reflect diet, not adaptation. *Journal of Evolutionary Biology*, 25: 873–880 <https://doi.org/10.1111/j.1420-9101.2012.02476.x> [IF₂₀₁₂ = 3.5; Q1; MNiSW points = 70]

After obtaining my PhD

1. Attisano, A., Tregenza, T., Moore, A. J., & Moore, P. J. (2013). Oosorption and migratory strategy of the milkweed bug, *Oncopeltus fasciatus*. *Animal Behaviour*, 86: 651–657 <https://doi.org/10.1016/j.anbehav.2013.07.013> [IF₂₀₁₃ = 3.1; Q1; MNiSW points = 140]
2. Kilner, R. M., Boncoraglio, G., Henshaw, J. M., Jarrett, B. J., De Gasperin, O., Attisano, A., & Kokko, H. (2015). Parental effects alter the adaptive value of an adult behavioural trait. *eLife*, 4: e07340 <https://doi.org/10.7554/eLife.07340> [IF₂₀₁₅ = 8.3; Q1; MNiSW points = 200]
3. Attisano, A., & Kilner, R. M. (2015). Parental effects and flight behaviour in the burying beetle, *Nicrophorus vespilloides*. *Animal Behaviour*, 108: 91–100 <https://doi.org/10.1016/j.anbehav.2015.07.020> [IF₂₀₁₅ = 3.2; Q1; MNiSW points = 140]

4. **Attisano, A.**, Murphy, J. T., Vickers, A., & Moore, P. J. (2015). A Simple Flight Mill for the Study of Tethered Flight in Insects. *JoVE (Journal of Visualized Experiments)*, 106: e53377 <https://doi.org/10.3791/53377> [IF₂₀₁₅ = 1.1; Q2; MNiSW points = 70]
5. Bojarska, K., Kuehn, R., Gazda, M. A., Sato, N. J., Okahisa, Y., Tanaka, K. D., **Attisano, A.**, Gula, R., Ueda, K., & Theuerkauf, J. (2018). Mating system and extra-pair paternity in the Fan-tailed Gerygone *Gerygone flavolateralis* in relation to parasitism by the Shining Bronze-cuckoo *Chalcites lucidus*. *PLoS ONE*, 13: e0194059 <https://doi.org/10.1371/journal.pone.0194059> [IF₂₀₁₈ = 2.8; Q2; MNiSW points = 100]
6. **Attisano, A.**, Sato, N. J., Tanaka, K. D., Okahisa, Y., Kuehn, R., Gula, R., Ueda, K., & Theuerkauf, J. (2018). Visual discrimination of polymorphic nestlings in a cuckoo-host system. *Scientific Reports*, 8: 1–10 <https://doi.org/10.1038/s41598-018-28710-5> [IF₂₀₁₈ = 4.0; Q1; MNiSW points = 140]
7. Gasperin, O. D., Duarte, A., English, S., **Attisano, A.**, & Kilner, R. M. (2019). The early-life environment and individual plasticity in life-history traits. *Ecology and Evolution*, 9: 339–351 <https://doi.org/10.1002/ece3.4749> [IF₂₀₁₉ = 2.4; Q2; MNiSW points = 100]
8. **Attisano, A.**, Thiel, F., Sato, N., Okahisa, Y., Bolopo, D., Tanaka, K. D., Kuehn, R., Gula, R., Ueda, K., & Theuerkauf, J. (2019). Breeding biology of the fan-tailed gerygone *Gerygone flavolateralis* in relation to parasitism by the shining bronze-cuckoo *Chalcites lucidus*. *Journal of Ornithology*, 160: 91–103 <https://doi.org/10.1007/s10336-018-1592-6> [IF₂₀₁₉ = 1.3; Q2; MNiSW points = 140]
9. **Attisano, A.**, Groß, L. B., Sato, N. J., Okahisa, Y., Tanaka, K. D., Gula, R., Ueda, K., & Theuerkauf, J. (2020). Impact of brood parasitism and predation on nest survival of the fan-tailed gerygone in New Caledonia. *Journal of Avian Biology*, 51: e02476 <https://doi.org/10.1111/jav.02476> [IF₂₀₂₀ = 2.4; Q1; MNiSW points = 100]
10. **Attisano, A.**, Hlebowicz, K., Gula, R., & Theuerkauf, J. (2021). Threat recognition and response in an avian brood-parasite host from New Caledonia. *Current Zoology*, 67: 255–262 <https://doi.org/10.1093/cz/zoaa061> [IF₂₀₂₁ = 2.7; Q1; MNiSW points = 100]
11. **Attisano, A.**, Sato, N. J., Tanaka, K. D., Okahisa, Y., Ueda, K., Gula, R., & Theuerkauf, J. (2021). Discrimination and ejection of eggs and nestlings by the fan-tailed gerygone from New Caledonia. *Current Zoology*, 67: 653–663 <https://doi.org/10.1093/cz/zoab066> [IF₂₀₂₁ = 2.7; Q1; MNiSW points = 100]
12. Theuerkauf, J., Villaviciencio, C. P., Adreani, N. M., **Attisano, A.**, Craig, A., D'Amelio, P. B., Gula, R., Lee, A. T. K., Montesana, L., Quillfeldt, P., Quirici, V., Quispe, R., Vasquez, R. A., Wingfield, J. C., & Masello, J. F. (2022). Austral birds offer insightful

- complementary models in ecology and evolution. *Trends in Ecology & Evolution*, 37: 759-767, <https://doi.org/10.1016/j.tree.2022.05.003> [IF₂₀₂₂ = 16.8; Q1; MNiSW points = 200]
13. **Attisano, A.**, Gill, B.J., Anderson, M.G., Gula, R., Langmore, N.E., Okahisa, Y., Sato, N.J., Tanaka, K., Thorogood, R., Ueda, K., Theuerkauf, J. (2023). Polymorphism at the nestling stage and host-specific mimicry in an Australasian cuckoo-host arms race. *Journal of Animal Ecology* 93: 30-43, <https://doi.org/10.1111/1365-2656.13849> [IF₂₀₂₃ = 3.5; Q1; MNiSW points = 140]
14. **Attisano, A.**, Anderson M.G., Langmore N.E., Gula, R., Theuerkauf J. (2025). Begging call mimicry and formation of host-specific lineages in the shining bronze-cuckoo, *Chalcites lucidus*. *Animal Behaviour*, 221:123083 <https://doi.org/10.1016/j.anbehav.2025.123083> [IF₂₀₂₅ = 2.3; Q1; MNiSW points = 140]
15. Theuerkauf, J., Bloc, H., **Attisano, A.**, Gula, R., Jourdan, H, Masello, J. (2025). Combining distance sampling and triangulation to estimate density of elusive rainforest vertebrates. *Biological Conservation*, 306:111133 <https://doi.org/10.1016/j.biocon.2025.111133> [IF₂₀₂₅ = 4.9; Q1; MNiSW points = 140]
16. Theuerkauf, J., Okahisa, Y., Hunt, G.R., Rouys, S., Boissenin, M., Bloc, H., Ugolini, D., Chartendraut, V., Duval, T., Angin, B., Desmoulins, F., Barré, N., Kuehn, R., Quin, D., **Attisano, A.**, Gula, R (2025). Density distribution of the flightless charismatic Kagu to inform conservation of endangered endemic rainforest species in New Caledonia. *Journal of Ornithology*, <https://doi.org/10.1007/s10336-025-02318-9> [IF₂₀₂₅ = 1.3; Q1; MNiSW points = 140]
17. Feeney, W.E., Kennerley, J.A., Wheatcroft, D., Liang, W., Lamb, J.B., Teunissen, N., Lawson, S.L., Enos, J.K., Zhou, B., Poje, C., Richardson, N.M., Ryan, T.A., Cowan, Z., Brooker, R.M., Attwood, M., Boersma, J., Zamora, M., **Attisano, A.**, Gula, R., Theuerkauf, J., Gloag, R., Fiorini, V.D., Gill, S.A., Peters, A., Honza, M., Spottiswoode C.N., Hauber, M.E., Manica, A., Webster, M.S., Blasi, D.E. (2025). Learned use of an innate sound-meaning association in birds. *Nature Ecology and Evolution*, <https://doi.org/10.1038/s41559-025-02855-9> [IF₂₀₂₅ = 14.1; Q1; MNiSW points = 100]
18. Perez-Granados, C., Funosas, D., Morant., J., Marin, O.H., Mendoza, I., Dehwal, A., **Attisano, A.**, Theuerkauf, J., Oliveira-Junior, D.D., Lima-Santos, C.S., Salustio-Gomes, C., da Paz, R.C., Pichorim, M., Goodale, E., Sebastián-González, E. (2025). Optimization of passive acoustic bird surveys: a global assessment of BirdNET settings. *Ibis*, <https://doi.org/10.1111/ibi.70013> [IF₂₀₂₅ = 1.8; Q1; MNiSW points = 100]
19. Pérez-Granados, C., Morant, J., Darras, KFA, Marín-Gómez, MG, Mendoza, I., Muñoz-

Mohedano, MA. **Attisano, A.**, Theuerkauf, J., E., Sebastián-González, E. (2025). WABAD: a world annotated bird acoustic dataset for passive acoustic monitoring. *Ecology*, in press [IF₂₀₂₅ = 4.4; Q1; MNiSW points = 200]

20. Funosas, D., Sebastián-González, E., Morant, J., Marín-Gómez, MG,, **Attisano, A.**, Theuerkauf, J., Goodale, E., Darras, KFA, Pérez-Granados, C. (2026) A global assessment of BirdNET performance: differences among continents, biomes and species. *Ecological Indicators*, 182: 114550, <https://doi.org/10.1016/j.ecolind.2025.114550> [IF₂₀₂₅ = 7.4; Q1; MNiSW points = 200]

10. List of participation in research teams implementing projects other than those specified in point II.4.

- 1) Dates: 02.03.2021 – ongoing

Role: collaborator

Funding: Fundacja SAVE

Project title: Monitoring and conservation of the wolf *Canis lupus* in central Poland

- 2) Dates: 01.01.2023 – 01.01.2024

Role: collaborator

Funding: Province Sud, New Caledonia

Project title: Establishment of a count method for the assessment of population size of the endangered *Ducula goliath*

IV. SCIENTOMETRIC DATA

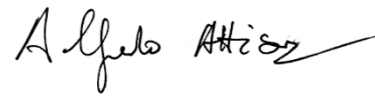
Data from 22/01/2026

1. Total value of Impact Factor of published articles: 95.05
2. Proportions of Journal quartiles: 77% Q1, 18% Q2, 5% Q3
3. Number of citations of the applicant's publications
 - a. Web of Science: 230 (with self-citations), 198 (without self-citations)
 - b. Scopus: 224 (with self-citations), 192 (without self-citations)
 - c. Google Scholar: 341
 - d. ResearchGate: 301
4. h-index
 - a. Web of Science: 9
 - b. Scopus: 9
 - c. Google Scholar: 9

d. ResearchGate: 10

5. Total number of points awarded by the MNiSW: 2900

(applicant signature)

A handwritten signature in black ink that reads "Alfons Attias". The signature is written in a cursive style with a long horizontal stroke at the end.

SCIENTIFIC REPORTS

OPEN

Visual discrimination of polymorphic nestlings in a cuckoo-host system

Alfredo Attisano¹, Nozomu J. Sato², Keita D. Tanaka³, Yuji Okahisa⁴, Ralph Kuehn^{5,6}, Roman Gula¹, Keisuke Ueda⁴ & Jörn Theuerkauf¹

Mimicry by avian brood parasites favours uniformity over variation within a breeding attempt as host defence against parasitism. In a cuckoo-host system from New Caledonia, the arms race resulted in both host (*Gerygone flavolateralis*) and parasite (*Chalcites lucidus*) having nestlings of two discrete skin colour phenotypes, bright and dark. In our study sites, host nestlings occurred in monomorphic and polymorphic broods, whereas cuckoo nestlings only occurred in the bright morph. Irrespective of their brood colour, host parents recognised and ejected parasite nestlings but never ejected their own. We investigated whether host parents visually recognised their own nestlings by using colour, luminance and pattern of multiple body regions. We found that the parasite mimicked multiple visual features of both host morphs and that the visual difference between host morphs was larger than the difference between the parasite and the mimicked host morph. Visual discrimination alone may result in higher chances of recognition errors in polymorphic than in monomorphic host broods. Host parents may rely on additional sensorial cues, not only visual, to assess nestling identity. Nestling polymorphism may be a trace of evolutionary past and may only have a marginal role in true-recognition of nestlings in the arms race in New Caledonia.

The interactions between brood parasites and their hosts are a classic example of a co-evolutionary process in which adaptation on one side leads to counter-adaptation on the other and so on¹. Phenotypic polymorphism is an example of such an adaptation with an important role in the host-parasite co-evolutionary arms race². For example, European Cuckoo *Cuculus canorus* females have two alternative plumage phenotypes that can help evading detection by the host^{3–5}, while polymorphism in egg appearance may allow hosts to escape brood parasitism^{6,7}. The evolution of successful host defences against brood parasitism, which involve the ability to detect and discriminate the brood parasite, is the fundamental driver in the arms race¹. Thus, cognitive and perceptual abilities of the host are essential in the evolution and maintenance of phenotypic variation in both the host and parasite². Variation in egg colour and pattern is an example of such defence strategy. In response to egg discrimination by hosts, brood parasites evolve egg mimicry^{8–12}, which in turn favours the evolution of distinct egg signatures^{13–15} or distinct polymorphism in host clutches^{6,7,16,17}. However, in a given host population, the success of such a defence strategy depends on phenotypic variation among clutches, which makes it difficult for the parasite to target a particular egg type, and on egg uniformity within clutches, which allows host parents to better discriminate a parasite against their own egg template^{18–23}.

In some host-cuckoo systems the arms race has reached the nestling stage, and host parents discriminate parasite nestlings from their own^{24–33}, which led to improved mimicry by cuckoo nestlings^{34,35} (but see³⁶). In theory, the same mechanisms that determine egg polymorphism should also apply to nestling polymorphism² and a recent study showed that polymorphism does indeed occur at the nestling stage in a cuckoo-host system in New Caledonia³⁷. In this Pacific island, the local subspecies of the Fan-tailed Gerygone, *Gerygone flavolateralis flavolateralis*, is the exclusive host of the local subspecies of the Shining Bronze-cuckoo, *Chalcites lucidus layardi*. Mimicry by bronze-cuckoo nestlings is common in *Chalcites*–*Gerygone* systems^{34,38} and it also occurs in New Caledonia³⁷. Despite the mimicry, two Australian *Gerygone* species^{25,26} and the Fan-tailed Gerygone from New

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Caledonia³⁷ are able to recognise and eject the cuckoo nestling from their nest before it can evict any host egg or nestling.

In contrast to Australian *Gerygone* species, Fan-tailed *Gerygone* have two distinct nestling morphs, with a pinkish-grey (bright) or a darkish-brown (dark) skin, which can occur in monomorphic and polymorphic broods^{37,39}. Brood colouration can vary within broods of the same parents, however it is most often constant for the same pair across multiple breeding attempts and extra-pair copulations do not influence the proportions of bright, dark or polymorphic broods³⁹. The Shining Bronze-cuckoo from New Caledonia also has two nestling morphs, dark and bright³⁷. Two nestling morphs, in this case however yellow and black, are also known to occur in the Australian Shining Bronze-cuckoo subspecies *C. l. plagosus*³⁴. In general, nestling polymorphism is rare in birds⁴⁰ and its occurrence within a brood parasite-host arms race is likely the result of strong selective pressure. Polymorphism in Shining Bronze-cuckoo nestlings may be an adaptation to mimic different hosts³⁴, whereas the two host nestling morphs may have originated as a host defence strategy caused by the strong selection imposed by parasitism.

Polymorphic broods in a cuckoo host increase phenotypic variation and contradicts the principle of intra-nest uniformity²³. This in theory should increase the likelihood of recognition errors by host parents. Within monomorphic broods, a cuckoo nestling might be accepted only if it matches the host nestling appearance and ejected if it does not, whereas in polymorphic broods a cuckoo nestling might always be accepted, because it matches one or the other host nestling morph. Moreover, in non-parasitised polymorphic broods, both host nestling morphs might sometimes be mistaken for a parasite and ejected from the nest. If nestling appearance is a cue for the recognition of the parasite, then polymorphic broods should decrease host fitness due to high chances of recognition errors. In our study sites, we only encountered the bright cuckoo morph and Fan-tailed *Gerygone* parents recognised and ejected the cuckoo nestling in both bright and dark monomorphic broods³⁷. However, in our former research³⁷, we were not able to confirm ejection of the parasite in polymorphic broods because none of the polymorphic broods in the previous study was parasitised.

It is also unclear how Fan-tailed *Gerygone* parents recognise the parasite nestlings. Some hosts of bronze-cuckoos discriminate the begging calls of the parasite from their own nestlings^{24,29}. Although vocal learning can start in the pre-hatching period²⁹, host parents can only discriminate nestlings once they emit begging calls with a definite structure, which usually forms around the age of 3–4 days^{24,29,38,41}. Fan-tailed *Gerygone* host parents usually eject the parasite within 24 hours from hatching³⁷ thus a similar auditory recognition based on properly structured nestling begging calls seems unlikely. Visual cues may be the main source of information for recognition. Fan-tailed *Gerygone* nestlings are covered by a dense layer of white multi-barbed down distributed over the head, back, rump, thighs and side areas, whereas Shining Bronze-cuckoo nestlings from New Caledonia have a sparser bristle-like down over the head, rump, thighs and side areas plus two dense layers of short white multi-barbed down around the orbital area³⁷. The bristle-like down is unusual for parasite cuckoo nestlings⁴² and absent in the Australian Shining Bronze-cuckoo subspecies³⁴, but bronze-cuckoos species that parasitise *Gerygone* hosts may present sparse down over some body areas^{26,34,43}.

In this study, we investigated if visual cues are sufficient for host parents to obtain reliable information regarding nestling identity. Visual recognition of nestlings in the New Caledonian cuckoo–host system is a challenging cognitive task because host parents must recognise a parasite in either a monomorphic or a polymorphic brood and recognise the two host nestling morphs in any brood as their own offspring. Thus, visual cues alone may allow nestling recognition in all type of broods if, based on one or multiple visual features, the two host nestling morphs appear more similar to each other than to a parasite nestling. On the other hand, visual cues alone may not be sufficient to avoid recognition errors if, based on one or multiple visual features, the two host morphs appear more different to each other than to a parasite nestling. In this case, the recognition of the parasite may involve other sensorial cues, for example auditory or olfactory, or a combination of multiple sensorial cues.

We analysed the visual information available to Fan-tailed *Gerygone* parents from newly hatched parasite and host nestlings. This represents the time when the ejection occurs under natural conditions (usually within 24 hours from hatching). We quantified skin colour and luminance, nestling dorsal region colour and luminance (with skin and down combined as a unique visual stimulus), colour and luminance of rectal flanges and the influence of down on nestling visual pattern and contrast. We then compared the measured values between host morphs and cuckoo nestlings and between bright and dark host morphs. Finally, we quantified age-related changes in skin luminance of the two host nestling morphs by measuring skin luminance from hatching until the appearance of juvenile plumage. The main objectives of our study were to (1) verify that ejection of the parasite occurs in any type of *Gerygone* brood, (2) assess the visual mimicry of the host morph by cuckoo nestlings, (3) quantify the effect of down on the overall colour, luminance, pattern and contrast compared to bare skin areas, (4) identify which visual cues may convey “host” vs. “non-host” information and (5) measure changes in skin colouration of host nestlings over time.

Methods

Study area and field methodology. We conducted field work at four sites on the main island (Grande Terre) of New Caledonia during six breeding seasons (September–January) in 2011/2012–2015/16 and 2017/2018: Parc provincial des Grandes Fougères (main study site) and near surroundings (21°37′39.44″ S, 165°45′41.75″ E), approx. 40 km west (21°35′58.89″ S, 165°23′55.61″ E) of the main study site, approx. 90 km southeast (22°9′36.96″ S, 166°25′24.11″ E) of the main study site and approx. 130 km northwest (20°41′45.55″ S, 164°59′38.41″ E) of the main study site. Field sites included areas of tropical rainforest (main study site) and savannah composed of grasslands and small patches of secondary forest (other sites). We located Fan-tailed *Gerygone* nests by following host parents returning to their nests. We found 190 active (containing at least one egg) nests of Fan-tailed *Gerygone*s of which 30 contained cuckoo eggs, resulting in an overall parasitism rate of 16%. It is unlikely that the parasitism rate was influenced by early ejection of the cuckoo egg as the Fan-tailed

Gerygone always accepted cuckoo eggs during our study, similar to other *Gerygone* hosts^{44,45}. Upon finding an active nest, we monitored egg development by candling until the day of hatching. Due to high predation rates, we enclosed nests with eggs older than 7 days in chicken-wire fences, which allowed host parents to pass through, but kept the nest out of reach of the main avian predators. We took photos of nestlings on the day of hatching (defined as day 0). Fan-tailed *Gerygone* parents rarely abandon their own nestlings but may abandon an empty nest. Therefore, we always removed one nestling at a time from the nest. In case of a single nestling, one person stayed at the nest to prevent parents returning to the empty nest while another took the photos. No pair abandoned an active nest at the nestling stage due to this manipulation. We limited handling of nestlings to 3 minutes and returned *Gerygone* nestlings to their nest immediately after taking the photos.

Because host parents usually ejected cuckoo nestlings soon after hatching³⁷, we replaced the cuckoo eggs of 8 nests with model eggs resembling size and colour of the real cuckoo eggs. We then artificially incubated the cuckoo eggs and took photos of the hatchlings. We returned the artificially incubated cuckoo hatchlings into their nests of origin and video recorded the behaviour of host parents. If the original nest was predated, we introduced the cuckoo hatchling into a suitable active non-parasitised Fan-tailed *Gerygone* nest and recorded the behaviour of host parents. In these cases, we introduced an artificial cuckoo egg in the non-parasitised nest a few days before and successively exchanged it with the cuckoo hatchling. Artificially incubated cuckoo hatchlings were always returned to or introduced in the host nests on the day of hatching.

Nestling photos. During four field seasons (2013/14, 2014/15, 2015/16, 2017/18), we took photos of *Gerygone* and cuckoo nestlings on the day of hatching. The two Fan-tailed *Gerygone* nestling morphs are easily distinguishable at hatching, thus we categorised them as bright or dark by visual inspection, whereas we only found cuckoo nestlings of the bright morph in our field sites. Nests of Fan-tailed *Gerygone* are dome shaped and with a narrow entrance, which restricted the possibility of taking standardised photos of the nestlings inside the nest. We therefore placed the nestlings in a shaded area outside the nest to avoid heat shock by direct sunlight exposure and took the photos using natural illumination. Each nestling was placed in a standardised natural resting position into a rectangular open box (50 × 20 × 10 mm) padded with cotton and lined with a grey photographic mat background (ca. 18% reflectance). We placed the camera approximately 40 cm vertically above the nestling to include head, nape, back and rump areas. Each photo included a 98% optical PTFE white reflectance standard (Ocean optics WS-1). During the 2013/14 and 2014/15 field seasons we used an Olympus E-5 digital camera equipped with a Zuiko 35 mm macro lens and took photos using standardised settings (shutter priority mode, ISO 200, 1/60). We used these photos for visual analysis of nestlings in the human visible (VIS) light spectrum (400–700 nm). During the 2015/16 and 2017/18 field seasons, we took photos with a Fuji IS-PRO ultraviolet (UV) sensitive digital camera equipped with a Coastal Optic 60 mm UV macro lens (Coastal Optic Systems). We took ultraviolet photos using a UV pass filter (Baader UV filter, transmitting between 320 and 380 nm), and VIS photos using a UV and infrared (IR) cut filter (Hoya UV & IR cut filter, transmitting between 390 and 700 nm). A custom-built filter holder allowed quickly swapping UV and VIS filters on the camera lens between shots of each nestling. We used a Nikon SB-80DX flashlight unit with the UV filter removed when taking UV photos. This provided additional ultraviolet light, decreased the exposure time for the UV photos, avoided blurred images and decreased manipulation time of nestlings. We used standardised camera settings for the Fuji IS-Pro in all photos (VIS: ISO 100, f8, variable shutter speed in aperture priority mode; UV: ISO 100, f8, 1/125).

Colour, luminance and pattern analyses. We linearised the red, green and blue camera sensor responses, standardised the digital photos in respect to light intensity using the pixel values of the 98% white standard and transformed pixel values to reflectance values⁴⁶. Over-exposed or not correctly linearised photos were excluded from the analysis. We aligned, scaled and combined images from 2015/16 and 2017/18 to multispectral images including the UV and VIS visual information. Based on the spectral sensitivities of the Fuji IS-Pro UV camera, we converted the multispectral images from camera colour space to relative photon catches of avian shortwave (SW), mediumwave (MW), longwave (LW) and UV sensitive cone photoreceptors. Both the Shining Bronze-cuckoo and the Grey Warbler *Gerygone igata* from New Zealand, a close relative of the Fan-tailed *Gerygone* from New Caledonia, have a violet sensitive (VS) visual system⁴⁷. We therefore used the spectral sensitivity of the most commonly used violet sensitive bird model, the peafowl *Pavo cristatus*⁴⁸.

Following the methodology described by Troscianko & Stevens⁴⁹, we generated polynomial models that translated the camera sensor response into peafowl cone-catch quanta by comparing the predicted camera and peafowl responses to a list of thousands of natural reflectance spectra. From calibrated multispectral images, we selected four regions per each focal nestling using the polygon selection tool in ImageJ⁵⁰. We selected each region to include the most evident visual features of the nestling that host parents might use to recognise nestlings. For each region, we generated an average photon catch value (amount of photons that reach the retina) according to the cone response of the peafowl visual system. This allowed us to quantify how Fan-tailed *Gerygone* host parents perceive the visual features of nestlings.

We selected two regions of bare skin from head and rump of the focal nestling and averaged the values to a single *skin* value. The third region, *dorsum*, included the entire dorsal region of the nestling in a natural resting position and thus combined bare skin and down. The fourth region, *flanges*, included the lateral rictal flanges of the focal nestling. We measured colour and luminance of all selected regions, but measured pattern differences using only the *skin* and *dorsum* regions. We then compared these values between pairs of nestling types (bright host, dark host, parasite). We performed the skin colour and luminance analysis on multispectral images of 22 nestlings (15 bright host, 4 dark host, 3 parasite). Colour differences were calculated using the log version of the Vorobyev – Osorio receptor noise model⁵¹, which generated “just noticeable differences” (JND) values. This predicts whether two colours are discriminable in sufficiently good light conditions based on the signal-to-noise

ratio of the focal visual system. A JND value below 1 means two colours are not discriminable, values between 1 and 3 mean two colours are difficult to discriminate except under optimal viewing conditions, values above 3 mean colours are increasingly easier to discriminate.

We measured achromatic (luminance) differences as described in Siddiqi *et al.*⁵². We calculated Weber fractions from the single cone ratios of the peafowl visual system (1: 1.9: 2.2: 2.1)⁴⁸ and used a noise-to-signal ratio of 0.05 for the most abundant cone type. We compared each individual nestling with all nestlings of a different type to obtain three groups of JND values: Cuckoo-Bright (cuckoo nestlings compared to the bright gerygone morph), Cuckoo-Dark (cuckoo nestlings compared to the dark gerygone morph) and Bright-Dark (bright compared to dark gerygone morph). Thus, our analysis included absolute comparisons of nestling pairs (JND values for each group) and relative differences between groups (JND comparisons between groups) in monomorphic and polymorphic host broods.

To measure the effect of down on the visual appearance of nestlings compared to bare skin areas, we used pattern difference analysis. We used VIS photos of 78 nestlings: 8 cuckoo, 59 bright and 11 dark host. We linearised images and converted them to reflectance values (RGB-equalised). However, we did not convert them into the predicted response of the peafowl visual system because we did not know the sensor spectral sensitivity of the Olympus camera. We thus obtained measurements as camera response objective values in the VIS spectrum (400–700 nm). We followed the Fourier analysis and bandpass filtering approach used in previous studies of animal markings and patterns^{53,54}. We generated pattern differences using a Fast Fourier Transform bandpass filter at 17 levels (from 2 pixels, increasing exponentially with $\sqrt{2}$ up to 350 pixels) and calculated spatial frequency differences between the two focal nestlings at each spatial scale by summing the absolute difference in energy. Such pattern difference describes the degree to which the nestling patterns match each other in terms of size, spacing and contrast. We used the same three groups as in the colour and luminance analysis to obtain the absolute comparison between pairs of nestlings (pattern difference value for each group) and relative differences between groups (pattern difference comparisons between groups).

In 2014/15 and 2015/16, we took photos of the ventral side of the focal nestling each day from day 0 (hatching day) until day 11. We chose the ventral side of the focal nestlings because it was featherless until the age of 13–14 days and thus allowed us to isolate skin areas to follow changes in skin luminance. We collected photos from 73 bright and 17 dark host nestlings. As we took photos with two different cameras, we only used objective reflectance measurements in camera colour space and linearised and standardised the photos relative to the 98% white standard. Markings and patterns are mainly encoded by achromatic information, thus we used the green camera sensor as this more closely corresponds to the avian luminance channel⁵⁵. We then converted measured reflectance values into proportions relative to the 98% white standard.

Statistical analyses. We ran mixed models to analyse the difference in colour JND, luminance JNDs and pattern difference within each region (*skin, flanges, dorsum*). We used GLMMs with a log link function to analyse colour and luminance JNDs and a LMM to analyse the pattern difference. We applied log transformation of the pattern difference values to achieve normally distributed residuals. The models included comparison (Bright-Dark, Cuckoo-Dark, Cuckoo-Bright) as main effect and site and pair ID as crossed random effects. We did not have cases of multiple breeding by the same pair (in the same or different years) in the used data set. Upon finding a significant effect, we ran a post-hoc pairwise comparisons between pairs of groups within each region. We quantified the rate of change in luminance during development of the two host morphs with an LMM for repeated measures that included site, nestling age, skin colour and the interaction age*skin as fixed effects and nestling ID (nested within age) as random effect. We used the Image Analysis Toolbox in ImageJ⁴⁹ to prepare the multispectral images, convert them into cone catches and obtain colour and luminance JNDs and pattern difference values. All statistical analyses were run in R 3.2.1⁵⁶.

Data availability. All data generated or analysed during this study are included as Supplementary Information files.

Ethical statement. The Province Sud of New Caledonia issued all permits (3045-2011, 2437-2012, 2532-2013, 2801-2014, 2476-2015, 2372-2017). Handling and collection of the nestlings was performed in accordance with the guidelines and regulations outlined in the permits. The 1st Warsaw Local Ethics Committee for Animal Experimentation approved the experimental procedures.

Results

Ejection of the parasite. In 30 parasitised breeding attempts, 15 cuckoo nestlings hatched, whereas in the other 15 cases the cuckoo did not hatch (3 cases), predators destroyed the nest (8) or we could not follow the nest until hatching (4). In all 15 cases of cuckoo hatching, the host parents ejected the parasite within 24 hours from hatching in all brood types (Table 1). We never observed Fan-tailed Gerygone host parents ejecting a Shining Bronze-cuckoo egg or one of their own nestlings in any brood type. We observed 9 cases of nest desertion at the egg stage (5% of 190 active nests) in 8 unparasitised nests and in 1 parasitised nest. The unparasitised nests were deserted in 7 cases due to unintended disturbance caused by the nest checks or placing of the video cameras and once after disturbance by predators. The parasitised nest was deserted 1 day after host parents ejected the cuckoo nestling, most likely as a consequence of human disturbance (placement of the protective fence) rather than parasitism. Thus, none of the nest desertion we observed seemed to be a direct consequence of brood parasitism, but rather an involuntary consequence of our research activities.

Successful parasitism was too rare to be detected with only 30 parasitised and monitored breeding attempts, which means that in our sites the parasite success should be lower than 3% (i.e. 1/30). We however observed four

Brood type	Number of broods	Parasitised nests	Cuckoo nestlings	Ejection events**
Bright	66	7	7	7
Dark	13	3	3	3
Polymorphic	8	1	1	1
unknown brood type*	104	19	4	4
	191	30	15	15

Table 1. Total number of Fan-tailed Gerygone broods, nests parasitised by Shining Bronze-cuckoo, number of cuckoo hatchlings and number of parasite nestling ejection events observed during the period 2011/12–2015/16 and 2017/18. *We could not determine the brood phenotype due to predation, hatching failure or accidental damage of the eggs. **In 11 naturally parasitised nests (5 bright monomorphic broods, 2 dark monomorphic, 1 polymorphic and 3 broods of unknown nestling composition) and in 4 nests (2 bright, 1 dark and 1 unknown) to which we introduced artificially incubated cuckoo nestlings.

cases of successful parasitism at other sites: one observation of a cuckoo nestling (about 11 days old) and three observations of a cuckoo fledgling fed by both Fan-tailed Gerygone host parents. In none of these four cases, we could determine the original host brood phenotype or if the cuckoo nestling had evicted host eggs or nestlings. Cuckoos hatched earlier than host eggs but gerygone parents did not always eject the cuckoo nestling before their own offspring hatched. Thus, Shining Bronze-cuckoo nestlings coexisted with Fan-tailed Gerygone nestlings in 6 broods (4 bright, 2 dark and 1 polymorphic) before being ejected by host parents.

Colour and luminance differences. Cuckoo nestlings mimicked the colour of both host morphs (Fig. 1a). The bright and dark host morphs had a relatively larger colour difference in the *skin* region than the cuckoo and both host morphs (Fig. 1a). All nestling groups had similar colour differences in the *dorsum* and *flanges* regions (Fig. 1a). The colour of the *skin* region was generally discriminable among nestlings in good light conditions whereas mean colour JND values were around the discrimination threshold of 3 JNDs for all nestling groups in the *dorsum* and *flanges* regions (Fig. 1a). The presence of down decreased the colour differences compared to bare skin areas (*dorsum* vs. *skin*, mean JNDs \pm 95% CI: 3.16 ± 0.30 vs. 5.09 ± 0.46).

Cuckoo nestlings mimicked skin luminance of both host morphs, in particular the bright morph, whereas the bright and dark host morphs had a relatively larger skin luminance difference than the cuckoo compared to both host morphs (Fig. 1b). Cuckoo nestlings were also more similar to dark host morphs in the *dorsum* region (Fig. 1b). Down of nestlings decreased achromatic differences compared to bare skin areas (*dorsum* vs. *skin*, mean JNDs \pm 95% CI, 7.48 ± 0.79 vs. 11.54 ± 1.49). There was no difference between nestling groups in the *flanges* region (Fig. 1b). With the exception of the *flanges* region, all mean luminance JND values were above the discrimination threshold of 3 JNDs for all groups within each region.

Skin of bright Fan-tailed Gerygones darkened as the nestlings grew older, whereas the dark nestlings kept their luminance (Linear mixed model, age*skin, $n = 90$, Wald $\chi^2 = 5.32$, d.f. = 1, $P = 0.021$; Fig. 2).

Pattern differences. The presence of down increased the pattern difference between nestlings in the *dorsum* region compared to the bare areas of the *skin* region (Fig. 3). Within the *dorsum* region, cuckoo chicks had lower pattern differences with bright host morphs than with dark host morphs (Fig. 3). Moreover, bright host morphs had a similar pattern difference with cuckoo chicks and dark host morphs (Fig. 3). There was no pattern difference between groups in the *skin* region (all pairwise comparisons $P > 0.1$; Fig. 3).

Discussion

We found that visual mimicry by Shining Bronze-cuckoo nestlings from New Caledonia was based on the mimicking of multiple visual features of the host, similar to bronze-cuckoos from Australia^{34,42}. We expected an improved mimicry of the respective similar host morph, i.e. bright cuckoo nestlings would better mimic bright host nestlings and dark cuckoo nestlings would better mimic dark host nestlings. However, we found only a bright cuckoo morph with features that improve mimicry with both host morphs rather than expressly mimicking only the bright host morph. The most conspicuous of these features is the presence of the white down. Host parents may use these features to visually recognise their nestlings, but cuckoo nestlings might also evade detection by the host because they are more similar to either host morphs when down and skin are combined as a single visual stimulus. More specifically, the down makes bright cuckoo nestlings more similar to both host morphs in terms of colour and more similar to dark host morphs in terms of luminance. The down also creates a high visual contrast with bare skin areas that increases the cuckoo mimicry with the bright host morph, whereas the dark morph has a higher contrast difference with both the cuckoo and the bright host morph. Similar to other brood parasites^{57,58}, cuckoo nestlings mimic the colour and luminance of the rectal flanges of host nestlings, as these are almost completely indistinguishable among all nestlings.

Although the Shining Bronze-cuckoo from New Caledonia parasitises a single host, it seems to have a mimicry strategy comparable with a generalist brood parasite. Its bright nestlings have features that closely resemble the preferred host, in this case the more frequent bright morph, but also general features that allow exploiting a “secondary host”, in this case the dark morph of the same host species. Generalist brood parasites exploiting different hosts are known to employ “average appearance” strategies. For example, genetic races (gentes) of the Common Cuckoo mimic eggs of their favourite hosts but also have “average” egg appearance that can facilitate

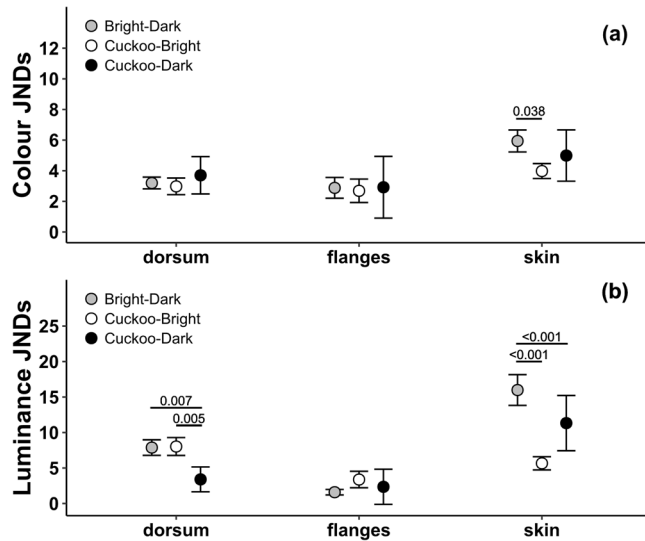


Figure 1. Colour (a) and luminance (b) just noticeable differences (JND, mean \pm 95% CI). Horizontal bars with P values indicate pairwise comparisons between groups or regions. Higher JND values indicates higher colour differences in the eyes of host parents between nestling types in each group. Sample size (number of comparisons) for *dorsum* and *skin* regions are Bright-Dark = 60, Cuckoo-Bright = 45, Cuckoo-Dark = 12 and for the *flanges* region are Bright-Dark = 27, Cuckoo-Bright = 18, Cuckoo-Dark = 6.

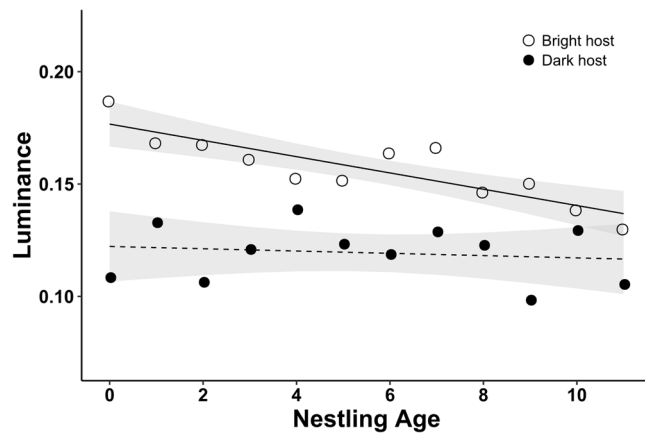


Figure 2. Regressions lines (with 95% CI as shaded area) of skin luminance in relation to age for the bright (solid line) and dark (dashed line) morphs of Fan-tailed Gerygone nestlings.

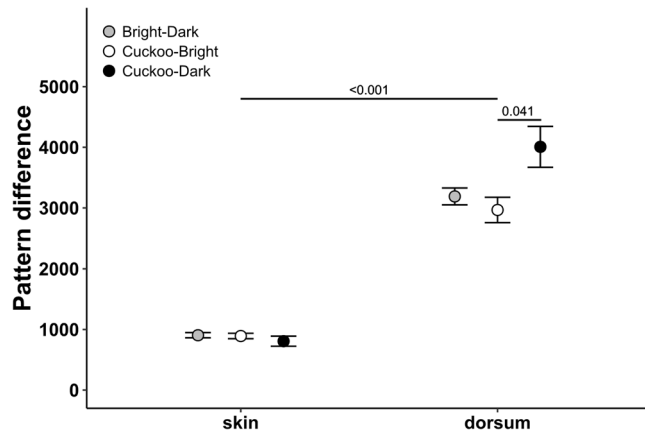


Figure 3. Mean pattern differences (pattern size, spacing and contrast) with 95% CI between nestling types in each group for each region of interest. Horizontal bars with P values indicate pairwise comparisons between groups or regions.

the use of secondary hosts⁵³. Similarly, the generalist Horsfield's Bronze-cuckoo *Chalcites basalis* lays eggs that are intermediate in appearance with the eggs of its various hosts⁵⁹. Nestlings of the Horsfield's Bronze-cuckoo also have a skin colouration that facilitates mimicry of their various host nestlings³⁴. The Shining Bronze-cuckoo subspecies from Australia, *C. l. plagosus*, has two nestling morphs that may depend on the presence of gentes specialised on particular hosts^{34,43}. In New Caledonia, the occurrence of possible gentes of the Shining Bronze-cuckoo subspecies *C. l. layardi* with bright or dark nestlings might depend on local frequencies of the host phenotypes. The relatively high frequency of bright host broods in our study sites may thus have led to low frequencies, or even disappearance, of the dark cuckoo morph which would explain why we never found a dark cuckoo morph during our study. In this case, there would only be selection acting on an average appearance of the bright cuckoo morph rather than on the frequencies of two contrasting cuckoo morphs.

The two host nestling phenotypes differ from each other in skin colour and luminance. In most cases, the dark host morph was as different from the cuckoo nestling as it was from the bright host morph. Luminance of the rictal flanges appeared to be the only feature with low visual difference between the two host morphs thus offering the only visual signals that could potentially allow distinguishing host from parasite. However, the use of the rictal flanges as a discriminatory cue may prove unreliable as the comparisons of colour and luminance between host and parasite nestlings showed low or no differences, meaning that cuckoo nestlings may exploit this visual signal to their advantage. Despite the striking visual difference between the two host morphs, we confirmed that ejection of the parasite occurred in the two monomorphic and in the polymorphic broods, extending previous observations on the ejection behaviour in Fan-tailed Gerygone³⁷. A single host-specific visual signal is unlikely to be a reliable discriminator between "host" and "non-host" as none of the visual signals taken singly may offer certainty of reliable recognition. Rather, host parents must combine multiple visual features of the nestlings to achieve discrimination of the parasite. Nevertheless, the recognition of the parasite by host parents is easier in monomorphic broods. In this case, cuckoo nestlings should be accepted 1) if all visual features are perfectly matched or 2) if host parents have low discrimination ability for one or multiple visual features. In polymorphic broods, the recognition process is far more complex because in parasitised broods, the dark host morph is the odd-looking one and thus it should more likely be recognised as "non-host". Moreover, in non-parasitised polymorphic broods, the two host nestling morphs appear different from each other, thus one morph should theoretically be recognised as "non-host" and ejected. However, this did not happen during our research. This raises the question of how host parents identify both host morphs as their own offspring and do not eject one as "non-host" by mistake.

Several studies showed that egg ejector hosts are able to recognise the parasite by discordancy with the majority of the clutch. For example, host parents may compare size⁶⁰ or colour and pattern^{61–63} of a foreign egg against the majority of the eggs in the clutch. Alternatively, host parents can recognise the parasite via true-recognition by comparing eggs to a learned template^{64,65}. In some cases, the two strategies can be used in conjunction⁶⁶. A recognition by discordancy seems unlikely in Fan-tailed Gerygone because of its low average clutch size of 2 eggs³⁷ and because Shining Bronze-cuckoo females usually remove one of the host eggs before laying their own⁶⁷. In an average parasitised Fan-tailed Gerygone brood, the ratio between host and parasite is therefore 1:1, which makes it impossible to compare one nestling against a majority. In addition, because the two host morphs greatly differ from each other, a recognition by discordance may lead to the ejection of one of the host nestlings in polymorphic broods despite the presence of the parasite. Our observations that ejection of the parasite can occur before hatching of host nestlings suggest that Fan-tailed Gerygone parents achieve true-recognition via a comparison with a cognitive template. The true-recognition of the parasite in Fan-tailed Gerygone is thus a more refined behaviour than other forms of advanced discrimination described in various cuckoo hosts^{24,28,36}. True-recognition and ejection of a parasite mimicking host nestlings requires high cognitive abilities from host parents, which may explain why such host defence behaviour is rare³³. It will take further research to find out whether the template is innate or learned in Fan-tailed Gerygone parents (see^{24,41}).

Although visual cues may have an important role in the parasite recognition process, their reliability may be limited by low light conditions in the incubation chamber of dome-shaped nests compared to open cup nests⁶⁸. Thus, the true-recognition by Fan-tailed Gerygone host parents might be based on a combination of multiple sensory cues. Auditory cues may have an important role in the recognition of a foreign nestling. For example, Superb Fairy-wrens *Malurus cyaneus* can discriminate Horsfield's Bronze-cuckoo nestlings by the different structure of their begging calls^{24,41}. In addition, bronze-cuckoo nestlings mimic the begging calls of their main host^{38,41}. However, nestlings may not produce proper begging calls until 3–4 days of age^{38,41}, whereas Fan-tailed Gerygone host parents eject the parasite nestling within 24 hours from hatching. In addition, ejection can occur before hatching of the host nestlings thus host parents cannot always compare, or do not need to compare, begging calls of the parasite and host. Another possibility is that true-recognition involves olfactory cues. For example, European Magpies *Pica pica* can discriminate foreign eggs in their clutch via odour cues⁶⁹ and experimental evidence seems to indicate that at least some birds may be capable of odour self-recognition⁷⁰. Olfactory cues may be particularly useful in a dome-shaped nest where volatile compounds may concentrate in a restricted space. Low light conditions in the nest may thus favour the use of auditory and olfactory cues for discrimination when visual cues become unreliable.

Nestling polymorphism is a rare occurrence in birds⁴⁰, thus the polymorphism of host and parasite in the New Caledonia system likely originated because of strong selective pressures imposed by the arms race. Bronze-cuckoo nestlings are known for their plasticity to mimic the preferred host³⁴, similar to host-specific races of the Common Cuckoo mimicking eggs of different hosts in Europe and Asia⁷¹. On the other hand, host nestling polymorphism should rarely occur because it would increase the likelihood of committing recognition errors²³. To our knowledge, only two cases of nestling polymorphism in hosts of brood parasites have been described and both involve a *Gerygone* host: the Fan-tailed Gerygone³⁷ from New Caledonia and the Grey Gerygone⁷² from New Zealand. However, while the Fan-tailed Gerygone is a nestling ejector, the Grey Gerygone is neither an egg⁴⁴ nor a nestling Briskie, pers. comm. ejector. Other nestling ejecting *Gerygone* species seem to have only monomorphic broods

with either dark or bright nestlings^{25,26}. In the Fan-tailed Gerygone, there are two possible scenarios to explain the colour of nestlings at hatching. In the first scenario, the dark morph is ancestral and a down-regulation mechanism of the melanocortin system suppresses the melanin synthesis with a consequent appearance of a bright morph at hatching. In the second scenario, the bright morph is ancestral and an up-regulation mechanism of the melanocortin system causes a dark morph to appear at hatching. A recent phylogenetic analysis⁷³ shows that the Fan-tailed Gerygone is most closely related to *Gerygone* species with dark nestlings²⁶, and in this study, we show that the bright host morph progressively darkens with age, whereas no change occurs in the dark morph. This suggests that the dark host morph is ancestral and that cuckoos probably mimicked it in the initial phases of the arms race. Mimicry by Shining Bronze-cuckoo nestlings then probably promoted polymorphism in the host with the appearance of a bright host morph that increased in frequency in the population under negative frequency selection. This in turn likely promoted polymorphism in cuckoo nestlings with the appearance of a bright cuckoo morph. Thus, host nestling polymorphism was probably favoured as host defence in the first stages of the arms race if the same evolutionary mechanisms as for egg polymorphism apply to nestling polymorphism². However, egg phenotype solely depends on the mother⁷⁴, whereas nestling phenotypes depend on the genetic background of both parents⁷⁵. Thus, nestling phenotypes may change if parents exchange partners or incur in extra-pair copulations and this may strongly constrain the selective advantage of nestling polymorphism as a host defence. In the Fan-tailed Gerygone, extra-pair copulations had however no impact on the frequency of nestling or brood types³⁹. Nevertheless, polymorphic broods may have further promoted the arms race, which involves generalist mimicry by the parasite and true-recognition based on multiple sensorial cues by the host.

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Author Contributions

A.A., N.J.S., K.D.T., R.K., R.G., K.U. and J.T. designed the study. A.A., N.J.S. and Y.O. conducted fieldwork. A.A. ran the analyses and wrote the first draft. All co-authors revised the manuscript.

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Article

Threat recognition and response in an avian brood-parasite host from New Caledonia

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Abstract

Nest predation and avian brood parasitism are the main sources of nest failure in many passerine birds. Large predators threaten both brood and parents, whereas brood parasites pose only a danger to eggs or nestlings. The fan-tailed gerygone *Gerygone flavolateralis* from New Caledonia is subjected to high rates of nest predation by the New Caledonian crow *Corvus moneduloides* (responsible for about 20–40% of predation) and moderate rates of brood parasitism by the shining bronze-cuckoo *Chalcites lucidus* (parasitizing about 18% of nests), which also depredates nests that are too advanced for parasitism (13% of nests). To test if fan-tailed gerygones are able to discriminate predators from brood parasites, we presented 3 bird models at active gerygone nests: a brood parasite/small nest predator (shining bronze-cuckoo), a large nest predator (crow), and a small non-native bird (common chaffinch *Fringilla coelebs*), which is unknown to the gerygone, as a control. We assessed the response of adult gerygones to the presentation of each model by measuring the minimum approach distance, number of alarm calls, number of attacks, and time to first nest visit after the presentation (latency). Adult gerygones often attacked the cuckoo, approached but never attacked the chaffinch and always avoided the crow. Latency was shorter after an attack response and during brooding, but similar among models. We did not find any link between the cuckoo model presentation and later ejection of cuckoo nestlings. We conclude that adult fan-tailed gerygones discriminate between different models and respond accordingly to the level of threat, but do not show awareness of parasitism risk and increase of nestling ejection rates following exposure to the cuckoo model.

Key words: brood parasitism, enemy recognition, frontline defense, mobbing, nest defense, nest predation

Predation and brood parasitism are major sources of nest failure, thus many small passerines defend their nest against potential threats (Schmidt and Whelan 1999; Caro 2005). However, the impact of brood parasites and nest predators are different for nesting birds as brood parasites only threaten the brood, whereas a predator might also be dangerous to the adults. Therefore, threat recognition is crucial for the nest owners because it allows them to assess the relative risk and respond with a specific defense (Caro 2005).

Large reproductive losses caused by brood parasites to their hosts lead to a host–parasite co-evolutionary arms race (Davies 2015). Discrimination of the parasite eggs or nestlings is a defense in many hosts (Stokke et al. 2002; Moskát et al. 2008; Sato et al. 2010; Spottiswoode and Stevens 2010; Tokue and Ueda 2010; Attisano et al. 2018; Noh et al. 2018), however, an even more effective defense would be to prevent the brood parasite from approaching the nest and laying an egg altogether (Feeny et al. 2012). Several studies showed that some hosts mob experimental models of brood

parasites placed at the nest (Mclean 1987; Moksnes et al. 1991; Davies et al. 2003; Dyrzc and Hałupka 2006) and often the response suggests recognition of the brood parasites (Neudorf and Sealy 1992; Gill and Sealy 1996; Welbergen and Davies 2008; Feeney et al. 2015). Hosts that are exposed to cuckoo models can perceive a higher risk of parasitism leading to increased nest guarding (Medina and Langmore 2016a) or a higher rejection rate of foreign eggs (Moksnes et al. 1993; Davies et al. 1996) and parasitized broods (Langmore et al. 2009). Host discrimination of the parasite can be influenced by the local variation of exposure likelihood to brood parasites (Lindholm and Thomas 2000; Welbergen and Davies 2009) or by spreading information about the risk of brood parasitism among members of the social group (Feeney and Langmore 2013), neighboring conspecifics (Welbergen and Davies 2008) or other host species (Yu et al. 2019). Brood parasites might represent a higher risk for their host than just parasitism as some can also be important nest predators (Soler et al. 2017) and cause high reproductive losses (Arcese et al. 1996). Nest predation by avian brood parasites is a strategy to increase the chances of parasitism by forcing hosts to lay a new clutch after depredating their nests that were in an advanced developmental stage unsuitable for parasitism (Soler et al. 2017).

The body size difference between nest owners and either a predator or a brood parasite could also play an important role in the adoption of a specific defense response. For small passerines, nest predators are usually larger and better armed and thus might threaten both adult and offspring survival (Caro 2005). The difference in size is less pronounced between avian brood parasites and their hosts, particularly in brood parasites that specialize on a small range of hosts (Medina and Langmore 2016b). A brood parasite might be perceived by nest owners as a direct threat to nest survival but not as a danger to adult survival. If the nest owner recognizes the adult cuckoo at the nest as a parasite and associates it with the later occurrence of cuckoo eggs or hatchlings in the nest, we would expect higher rates of ejection or higher levels of other anti-parasite behavior.

In the remote archipelago of New Caledonia, the fan-tailed gerygone *Gerygone flavolateralis* is the sole host of the obligate brood-parasitic shining bronze-cuckoo *Chalcites lucidus* and their interactions resulted in an arms race that escalated to the nestling stage (Sato et al. 2015). Gerygone parents accept the cuckoo egg, despite being larger and odd-looking compared to gerygone eggs, but eject newly hatched cuckoo nestlings from their nest even though cuckoo nestlings mimic the appearance of the host nestlings (Attisano et al. 2018). Nests of fan-tailed gerygone suffer high rates of predation by native birds (about 45% of nests), in particular the New Caledonian crow *Corvus moneduloides* and the white-bellied goshawk *Accipiter haplochrous* (Attisano et al. 2020). The shining bronze-cuckoo parasitizes 18% of the nests on average, but it also depredates about 13% of the active nests by removing whole host clutches or broods (Attisano et al. 2020). A few gerygone species have been observed to mob a cuckoo at the nest (Briskie 2007; Gloag et al. 2014; Attisano et al. 2019). However, none of these observations clarifies if mobbing is due to recognition of the brood parasite per se or rather is a generalized response against a potential enemy approaching the nest.

In this study, we tested the anti-predator/parasite response of adult fan-tailed gerygones by presenting at the nest 3 bird models: a shining bronze-cuckoo as their brood parasite and at the same time small nest predator, a crow as a large nest predator that can also be a risk for adults, and a common chaffinch *Fringilla coelebs* as an

non-native unknown bird, which is neither a parasite nor a predator and thus used as a control. We quantified the perceived risk posed by the models for the nest owners by assessing the response to the respective models and measuring the time to first nest visit after the model presentation (latency). We expected the gerygone parents to react more aggressively toward the cuckoo and the chaffinch because they should perceive these models as less threatening than the crow. If gerygones perceive the cuckoo as a parasite, we would also expect to observe higher aggressiveness toward the cuckoo model during earlier stages of the breeding cycle, when the risk of parasitism is higher. Finally, we assessed if the anti-parasite defense of the gerygone, i.e. ejection of parasite nestlings, was associated with an earlier aggressive response to the cuckoo model.

Material and Methods

Field sites and study species

We conducted fieldwork at three sites on the main island (Grande Terre) of New Caledonia from October to December 2019: Parc des Grandes Fougères (PGF, 21°37' S, 165°45' E), Farino and surroundings (Farino, 21°39' S, 165°46' E) and Domaine de Deva (Deva, 21°35' S, 165°22' E). The field sites included areas of forest, thicket and savannah. The fan-tailed gerygone is a small insectivorous bird (adult mass 6.0–6.2 g, length 10 cm), which breeds from September to January with a peak in October/November (Attisano et al. 2019). The nest is dome-shaped and attached to small branches in trees, bushes, or ferns. Gerygone pairs are socially monogamous, form long-term bonds and use the same territory over several breeding seasons (Gazda et al. 2015; Bojarska et al. 2018; Attisano et al. 2019).

We searched for active nests by following adults flying to their nests or by directly searching for nests in known territories. Nests can be located at various heights (range 0.4–20 m), but for practical reasons, we restricted our study to nests lower than 3 m. Upon finding an active nest we recorded the GPS coordinates and determined the stage of the nest by egg candling (Lokemoen and Koford 1996) or by ageing nestlings based on their development stage. This allowed us to estimate the nest age by counting an average of 4 days for laying, 18 days of incubation, and 14 days of brooding (Attisano et al. 2019).

Experimental trials

We presented 3 bird models at active nests (containing at least one egg or nestling) of fan-tailed gerygones. In previous studies with similar experimental designs as ours, researchers used a single model of a brood-parasite (Moksnes et al. 1993; Dyrzc and Hałupka 2006; Langmore et al. 2012), 2 models (Neudorf and Sealy 1992; Gill and Sealy 1996; Davies et al. 2003) or multiple replicates of each model (Grim 2005; Welbergen and Davies 2008; Feeney and Langmore 2013; Feeney et al. 2015; Medina and Langmore 2016a; Yu et al. 2020). We decided to use a single replicate of each model and, to validate the assumption that models represented different levels of threat to adults and nests of fan-tailed gerygones, compared the response to models with the reaction to 9 nest approach events by live shining bronze-cuckoos and 12 predation events by live predators that we recorded by video monitoring of active nests from 2011 to 2019 (Attisano et al. 2020).

As models, we used a stuffed female shining bronze-cuckoo, a stuffed male common chaffinch and a plastic model of a crow resembling a New Caledonian crow with black feathers (goose,

New Caledonian crow) glued onto it (Figure 1). The shining bronze-cuckoo is smaller (mass 21–22 g, length 15–17 cm: Payne 2005) than the other major nest predators and is easily distinguishable from any other sympatric bird species due to its unique plumage coloration with golden green upperparts and barred brown belly. We used the chaffinch and the plastic crow model because we had no access to other stuffed species of New Caledonian birds. We used the common chaffinch (mass 17–29 g, length 14–18 cm: Clement 2020) as a novel and nonthreatening bird because it was similar in size to an adult shining bronze-cuckoo, but different in appearance to any New Caledonian passerine. The plastic crow model resembled overall size, color and appearance of a New Caledonian crow, which is one of the largest (mass 280–355 g, length 40–43 cm: Debus 2020) and most important nest predators of fan-tailed gerygones in New Caledonia (Attisano et al. 2020). Although we never observed predation of adult gerygones, New Caledonian crows are likely to depredate on fledglings, and their large size and predatory behavior might cause adult gerygones to see them as a direct threat to both the nest and their own survival.

We presented the models at 30 active nests (6 parasitized and 24 non-parasitized) during the incubation or brooding stage. Each nest was subjected to one experimental trial, except for 2 nests where we presented models once during incubation and once during brooding. We performed trials in the morning from 7:00 to 13:00 h between the 1st and the 14th day of incubation or between the 1st and 7th day after the first chick hatched. We did not conduct trials during laying as during this period fan-tailed gerygone parents spend little time attending the nest and are susceptible to disturbance which may lead to nest abandonment. Shining bronze-cuckoos can parasitize host nests during early incubation (Gill 1983), meaning this is still a sensitive period for the host and the reaction to a cuckoo should not differ from the laying period. In addition, we were also interested in the variation in response between incubation and brooding periods to understand if hosts were more aggressive toward the cuckoo during early breeding stages (more sensitive to parasitism) or during later stages (more sensitive to depredation; see Briskie and Sealy 1989; Gill and Sealy 1996).

We fixed models to a small wooden square base (5 × 5 cm for cuckoo and chaffinch, 8 × 8 cm for crow) and placed them with Velcro tape on a mount consisting of a larger square wooden base (14 × 14 cm) attached on top of a 1-m wooden pole (Figure 1). We approached the focal nests with the models concealed in a box. We then checked from a safe distance for the presence of adult birds around the nest and if the female was incubating or brooding. If the female was inside the nest, we observed the nest from a hidden location about 10 m away until the end of the incubation/brooding bout. Once the female left the nest and neither parent was around, we first placed the presentation mount (without models) at a distance of about 2 m from the nest, directly facing the entrance. We then observed the nest from the hidden location, waiting for the parents to return and become habituated to the presence of the mount and let the female to resume incubation/brooding. We considered the parents to be habituated to the mount when both adults approached the nest showing no agitated behavior, females resumed a normal incubation/brooding behavior, males showed no signs of alertness (no alarm calls, no approach to the mount) and when both parents left the nest with no sign of alertness when leaving for the successive foraging trip. Habituation to the mount took less than 1 min and the mount never caused an alert reaction by any of the parents.

The average interval female fan-tailed gerygones spend away from the nest is 14 min (Attisano et al. 2019). When the female left



Figure 1. Models on the mount used for the trials (from top to bottom): shining bronze-cuckoo, common chaffinch, crow.

the nest for the second time, we waited an additional 8 min before placing the first model on the mount to start the presentation trial. We left the mount in place and only swapped models between presentation trials. We randomized the presentation order of the models for each trial and presented the models at least one hour apart from each other to allow for any carry-over effect to be minimized. We presented each model on the mount for a maximum period of 10 minutes, however if gerygone adults responded to the model within this time interval, we limited the presentation time to 2 minutes from the start of the response to minimize stress for the parents and decrease the chances that a predator would notice the nest due to the additional activity. We video- and audio-recorded (Nikon D5100 with Nikkor 70-300mm f/4.5-5.6G IF-ED lens, digital recorder Tascam DR-05 with directional microphone Rode NTG-2) the reaction by the gerygones from the hidden location. We categorized the response to the models as “no approach” (parents staying > 5 m from the model, no mobbing), “approach”

(parents approaching < 2 m from the mount, no physical contact with the model, alarm calls) or “attack” (physical contact with the model, alarm calls). If the parents did not return to the nest within 10 min of model presentation, we removed the model and categorized the trial as “no response.” We defined latency as the period of time between the end of the presentation trial (model removed from the mount) and the first nest visit of one of the parents after the model removal. In total, we performed 95 presentation trials, 32 with the chaffinch, 32 with the cuckoo and 31 with the crow (heavy rain terminated one trial), during incubation ($n = 74$) or brooding ($n = 21$). We counted the number of attacks and alarm calls directed to the models during the 2-min presentation time using the video and audio recordings. We visualized the audio recordings of alarm calls in Raven Pro 1.6.1 (Center for Conservation Bioacoustics 2019) using spectrograms with a Hann window, 1000 samples, 63.4 filter bandwidth and 80% overlap. We isolated single alarm call phrases and counted number of elements in the phrase, total phrase duration, highest frequency, and peak power.

Response to models and nestling ejection

We checked if the exposure to the cuckoo model triggered a lasting response of gerygone parents leading to later nestling ejection. We ran presentation trials during incubation at 6 parasitized nests and checked the nests 1 day after hatching of the cuckoo for ejection or acceptance of the cuckoo nestling. In addition, we opportunistically presented the cuckoo model for 2 min at one nest containing an about 15-day old cuckoo nestling accepted by its foster parents and assessed the response of adult gerygones to the cuckoo model.

Statistical analyses

We ran all analyses in R 3.6.3 (R Core Team 2019). We considered only the cases for which we observed an active response to the models and excluded from the statistical analysis the “no response” trials and classified the response to models as an ordinal variable (Response) with values “no approach” (1), “approach” (2) and “attack” (3). We used Response as the dependent variable with the categorical variables Model (chaffinch, crow, cuckoo), Site (Deva, Farino, PGF), Stage (incubation, brooding) and Order (presentation order of the models coded as 1, 2 and 3) as fixed effects and the metric variables Nest Age (age of the nest when the trial was conducted, with 0 = day of first egg laid and 36 = fledging day, range during trials: 5–30) and Day Season (day of the season when the trial was conducted, with 1 = 1st October, range during trials: 3–74) as covariates to include temporal effects of nest age and season on the response to the bird models. We included Nest ID as random effects to control for individual variation in response. We also analyzed which factors influenced the latency to return to the nest following the model presentation. Some adult gerygones returned to the nest before removal of the model leading to negative values for Latency (ranging from –1.4 to 30.2 min). We therefore added the absolute value of the minimum Latency (1.4 min) plus 1 min to each Latency value to obtain a log-normal distribution. We used Latency as the dependent variable with Model, Site, Stage and Response as fixed effects and Nest ID as a random effect. The variable Response was not independent from the variables Model and Stage, thus to avoid collinearity effects we did not include models that included a combination of these factors. We built ordinal logistic regression mixed models with the function *clmm* from the package *ordinal* (Christensen 2019) to analyze Response and general linear mixed models with log-link function using the function *glmer* from the package *lme4* (Bates

et al. 2015) to analyze Latency. We built all models that included simple combinations of the main factors and ranked them based on their AICc values using the function *aictab* from the package *AICcmodavg* (Mazerolle 2019). We included all trials with active responses to models in the analyses, including the two nests at which trials were repeated at the incubation and brooding stage because the inclusion or exclusion of these nests did not change the results. Finally, we compared the number of alarm calls and attacks by linear mixed models using the number of attacks or alarm calls as the dependent variable, Model, Site and Stage as fixed effects, and Nest ID as a random effect. We report all means with 95% confidence intervals and percentages with Bonferroni confidence intervals (Byers et al. 1984).

Ethical standards

The Province Sud of New Caledonia issued the permit (2720-2019) for fieldwork. The Province Nord of New Caledonia allowed us to stuff a shining bronze-cuckoo found dead in Hienghène in 2011. The stuffed chaffinch model was obtained from the collection of the Ornithological Station of the Museum and Institute of Zoology, Polish Academy of Sciences.

Results

Response to models

Out of 95 trials, adult gerygones responded to the three bird models in 69 cases (73%). The response of adult gerygones strongly depended on the model type (Figure 2A, Supplementary Material S2). When excluding the “no response” cases, gerygones attacked the cuckoo model in $57 \pm 25\%$ ($n = 23$) of trials, whereas they never attacked the chaffinch ($n = 22$) or crow ($n = 24$) model. Gerygones approached the chaffinch model in $82 \pm 18\%$ ($n = 22$) of trials, which is more often than they did with the cuckoo ($26 \pm 22\%$, $n = 23$) or the crow model ($17 \pm 17\%$, $n = 24$). Gerygones avoided (“no approach”) the crow model in $83 \pm 17\%$ ($n = 24$) of trials, which is more often than they did with the chaffinch ($18 \pm 18\%$, $n = 22$) or the cuckoo model ($17 \pm 19\%$, $n = 23$). The behavioral response of gerygone pairs to the experimental models was consistent with what we observed during video monitoring of live shining bronze-cuckoos or predators approaching active nests. In the 9 cases of video monitored visits of female cuckoos to active nests, the gerygone parents either were not near the nest ($n = 7$) or attacked the cuckoo at the nest in the same way gerygone pairs did with the experimental cuckoo model ($n = 2$). In 7 cases of predation by New Caledonian crows and 5 cases of predation by the white-bellied goshawk, the adult gerygones did not alarm, approach or mob the predators.

The AICc ranking suggested a strong effect of Model on the response of adult gerygones whereas Stage was less important, and Order only had a minor effect (Table 1, Supplementary Material S2). Adult gerygones approached the models (all combined) more frequently during incubation ($47 \pm 17\%$, $n = 49$) than during brooding ($25 \pm 23\%$, $n = 20$), whereas they did not approach or attack the models more frequently during brooding (no approach: $50 \pm 27\%$; attack: $25 \pm 23\%$; $n = 20$) than during incubation (no approach: $37 \pm 16\%$; attack: $16 \pm 13\%$; $n = 49$).

Mobbing and nest fate

Gerygones produced a clearly higher number of alarm calls during the 2-min presentation trials in response to the cuckoo compared to

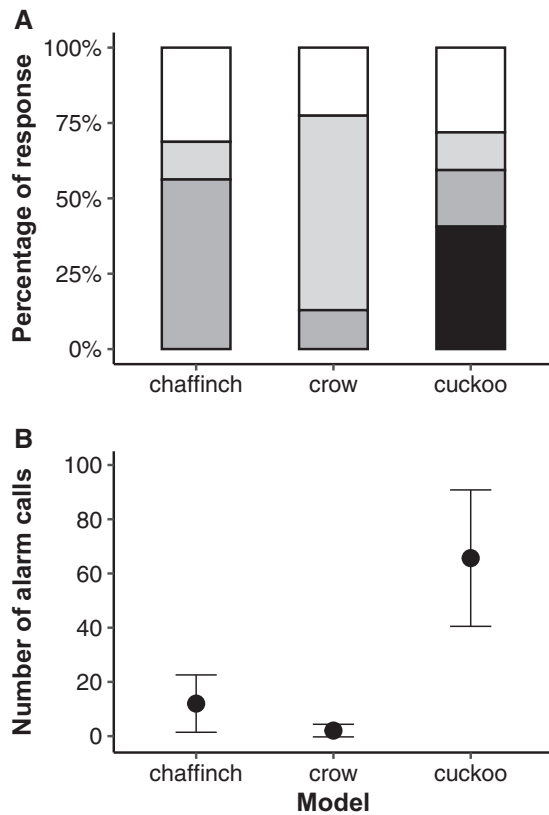


Figure 2. (A) Percentages of responses by fan-tailed gerygone adults to each model presented at active nests. Fillings in bars represent: Response: open = no response, light grey = no approach, dark grey = approach, black = attack (chaffinch: $n = 32$; crow: $n = 31$; cuckoo: $n = 32$). (B) Average \pm 95% CI number of alarm calls directed to the experimental models by adult gerygones during 2 min of presentation (chaffinch: $n = 22$; crow: $n = 24$; cuckoo: $n = 23$).

the other models (Figure 2B). They also attacked only the cuckoo model (mean number of attacks: 11 ± 5 , $n = 16$), whereas they swooped the chaffinch only occasionally and never approached the crow. Alarm calls of fan-tailed gerygones consisted of repeated phrases uttered in unison (39 ± 2 dB at a distance of 10 m, $n = 27$). There was no difference in the structure of alarm calls (phrase duration, number of elements in the phrase, highest frequency, peak power) to different models or type of response (Supplementary Material S2). Alarm calls to any of the models ($n = 30$) were given in 20% of the cases with no approach, in 37% with approach and in 43% with a direct attack (only to the cuckoo model). In 28 of the 30 mobbing cases, the nest owners alone responded to the model in front of their nest. In the two other cases at two different nests, conspecific neighbors were attracted by the alarm calls of the nest owners and briefly participated in mobbing the cuckoo model together with the resident pair. In both cases, the neighbor intervention was shorter (<20 s) and less intense (only approach and alarm, no attack) than that of the nest owners. We identified the individual that attacked the cuckoo model in 12 cases by colored plastic leg bands: in 6 cases only the male, in 1 case only the female and in 5 cases both sexes attacked the model.

We could determine the nest fate in 28 of the 30 nests: 19 were depredated, 3 were successful and 6 failed for other reasons (heavy rain, parasitism, dead chicks for unknown reasons). Nests at which parents mobbed at least one model during the trials, were not more often depredated ($59 \pm 27\%$, $n = 17$) than nests at which parents

Table 1. Models ranked by AICc for effect on response to bird models and latency of first nest visit by adult gerygones after the model removal ($n = 69$ trials, excluding “no response” cases)

Parameter in model	K	AICc	Δ AICc	w
Response				
Model	8	113.17	0	0.67
Model + Stage	7	115.33	2.16	0.23
Model + Order	7	117.75	4.58	0.07
Model + Stage + Order	8	120.06	6.89	0.02
Model + Site + Order	9	122.17	9.00	0.01
Model + Stage + Site + Order	10	124.82	11.65	0.00
Model + Stage + Site + Order + Nest Age + Day Season	12	128.97	15.80	0.00
Nest Age	4	152.71	39.54	0.00
Day Season	4	152.82	39.65	0.00
Stage	4	152.87	39.70	0.00
Site	5	153.27	40.10	0.00
Order	5	154.80	41.63	0.00
Latency				
Response	5	521.87	0	0.63
Response + Site	7	525.69	3.82	0.09
Stage	4	526.02	4.15	0.08
Model + Stage	6	526.33	4.47	0.07
Model	5	527.39	5.52	0.04
Nest Age + Day Season	5	527.68	5.81	0.03
Stage + Site	6	528.20	6.33	0.03
Model + Stage + Site	8	529.08	7.21	0.02
Site	5	530.52	8.65	0.01

did not mob any of the models during the trials ($82 \pm 26\%$, $n = 11$; Supplementary Material S2). Adult gerygones produced a similar number of alarm calls at nests that were depredated (65 ± 35 , $n = 19$) compared to nests that were successful (74 ± 56 , $n = 3$; Supplementary Material S2). No nest was abandoned due to presentation of the model.

Latency

Latency of the first nest visit after presentation tended to be shorter in parents that attacked the model (Figure 3A) and those that were brooding (Figure 3C), but was on average similar for the different models presented at the nest (Figure 3B). The model ranking indicated that Latency was mainly dependent on Response of adult gerygones, whereas the other parameters had low rankings (Table 1, Supplementary Material S2).

Cuckoo nestling ejection

In nests at which we presented models, 5 out of 6 cuckoo eggs hatched. Including the nest containing a 15-day old cuckoo nestling where we only presented the cuckoo model, we recorded 4 cases of ejection within 1 day from hatching and 2 cases of acceptance by the host parents (the 15-day old cuckoo nestling fledged later, the other was depredated when 16 days old). In both nests in which the gerygone parents accepted the cuckoo nestling, they attacked the cuckoo model. In the 4 nests where the gerygone parents ejected the cuckoo hatchling, they either attacked the cuckoo model (1 nest), only approached it (2 nests), or did not respond (1 nest).

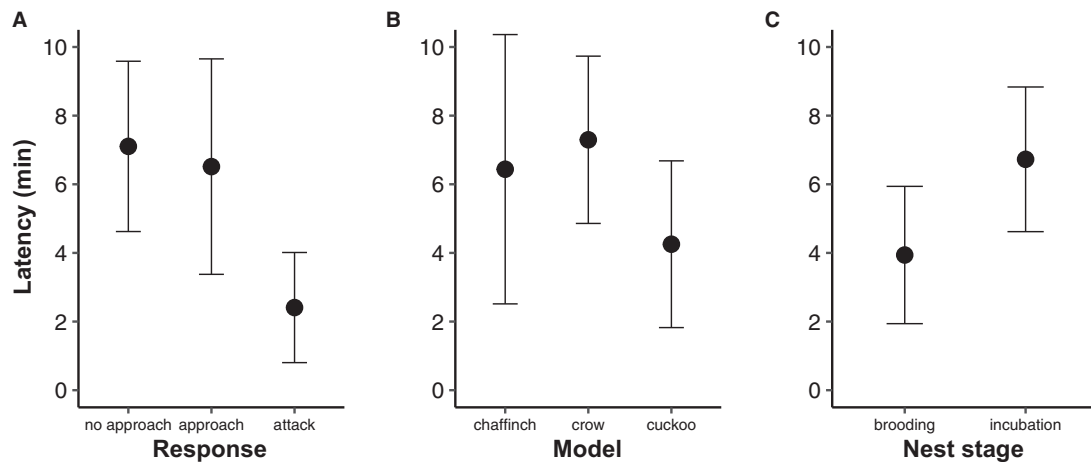


Figure 3. Latency to the first nest visit (mean \pm 95% CI) after removal of the model at the nest depending on (A) response to the model (no approach: $n = 27$; approach: $n = 27$; attack: $n = 12$), (B) model presented at the nest (chaffinch: $n = 21$; crow: $n = 23$; cuckoo: $n = 22$) and (C) nest stage (brooding: $n = 17$; incubation: $n = 49$).

Discussion

We found that fan-tailed gerygones discriminated among bird models presented at their nest and responded differently. Adult gerygones clearly recognized the cuckoo model as a threat as they usually attacked it, whereas they never attacked the chaffinch and always avoided the crow. This is consistent with the observation that many hosts of brood parasites discriminate among models presented at the nest (Gill and Sealy 1996; Welbergen and Davies 2008; Feeney et al. 2015). The discrimination ability of the fan-tailed gerygone is not surprising because the shining bronze-cuckoo has a very different appearance than any other New Caledonian bird and is smaller than other common nest predators on the island. This eliminates confounding effects such as resemblance to other sympatric birds (Grim 2005; Davies and Welbergen 2008; Trnka et al. 2012; Feeney and Langmore 2013). However, many hosts experiencing a brood parasite in the proximity of their nest show awareness to the risk of parasitism by increasing nest guarding (Medina and Langmore 2016a) or mobbing the brood parasite when vulnerability to parasitism is the highest during the nesting cycle (Briskie and Sealy 1989; Gill and Sealy 1996). We cannot unequivocally clarify whether the cuckoo is perceived only as a predator or also as a parasite by adult gerygones. However, we found no evidence that exposure to the adult cuckoo model caused anti-parasite responses by the gerygones, suggesting no awareness of parasitism risk. Therefore, our results rather suggest that the fan-tailed gerygone response to the cuckoo model is an immediate anti-predator defense.

Many hosts defend their nest by mobbing both brood parasites and predators (Gill and Sealy 1996; Lindholm and Thomas 2000; Avilés and Parejo 2006); however, fan-tailed gerygones often mobbed the cuckoo model but never even approached the crow model. The response to the crow model suggests that parents rather lower their activity around the nest to avoid nest detection by the predator (Martin et al. 2000). Because nest concealment has no effect on predation rates and parasitism in the fan-tailed gerygone (Attisano et al. 2020), being inconspicuous around the nest might be the best strategy against predation. However, the response to the cuckoo and chaffinch models also indicate that parents might take the risk of disclosing the nest in some cases, most likely because neither cuckoo nor chaffinch are perceived as dangerous to their own survival, whereas the crow likely is. The size difference between the

models may not be the sole factor affecting the risk assessment, as fan-tailed gerygones also approach and alarm when humans approach their nest, a behavior that in many cases helped us locating the nest. The “no approach” and “no alarm” response suggests therefore that crows are perceived as a danger for adults. The response to a potential nest predator depends on the trade-off between nest survival and adult survival (Montgomerie and Weatherhead 1988). Fan-tailed gerygones might prioritize nest survival if a cuckoo is approaching the nest, but own survival if a crow is approaching because mobbing a larger and better armed predator could result in injuries or death (Sordahl 1990). The survival of the parents should have priority over nest survival if a predator is perceived as dangerous for adults. The avoidance of the crow model was indeed consistent across nest age and stage, suggesting that adult survival was always prioritized over the survival of eggs or nestlings. Avoidance, mostly associated with presentation of the crow model, was also associated with a longer latency to the first nest visit after model presentation, implying that adult gerygones were more aware of a potential risk in these occasions.

Gerygones recognized the adult cuckoo model at the nest as a low threat to themselves as they attacked this model and had shorter latency to the first nest visit after an attack response. This is likely an anti-predator defense toward a nondangerous nest predator rather than an anti-parasite response. The shining bronze-cuckoo parasitizes fan-tailed gerygone nests mainly during the short laying period (4 days on average), but this is also the time when parents leave the nest unattended for the longest periods (Attisano et al. 2019). We observed several instances of cuckoos approaching nests during laying but never observed gerygone parents attacking the cuckoos at this stage. Nest parasitism can occur after host incubation has begun (Gill 1983); however, shining bronze-cuckoos are also major predators of fan-tailed gerygone nests during the incubation and brooding stages (Attisano et al. 2020). Therefore, cuckoos approaching the nest are attacked because they directly threaten the nest but are not dangerous for the adult gerygones. Similarly, pied flycatchers *Ficedula hypoleuca* would not attack a Eurasian sparrowhawk *Accipiter nisus* as it is dangerous for the adults, but attack great spotted woodpeckers *Dendrocopos major* as they endanger offspring but not adults (Dale et al. 1996). We did not find any indication that a mobbing response would increase the risk of nest

predation, thus attacking the cuckoo might not be a risky strategy to save the nest. In addition, the response to the cuckoo model did not differ between incubation and brooding, thus fan-tailed gerygone adults seem to perceive the cuckoo as a permanent predation threat across the nesting period. Briskie and Sealy (1989) also suggested that the aggressive response of least flycatcher *Empidonax minimus* towards brown-headed cowbirds *Molothrus ater* does not vary during the entire nesting period because cowbirds depredate on eggs and nestlings.

Finally, although based on a small sample size, we found no clear association between response to the cuckoo model and ejection of cuckoo nestlings. We suggest that the lack of such association is likely caused by the time lag between presentation of the adult cuckoo model and the hatching of the parasite nestling. As shown in egg ejector hosts, observing an adult cuckoo at the nest leads to high egg rejection rates of foreign eggs because these two stimuli occur within a short time period (Moksnes et al. 1993; Davies et al. 1996; Bártol et al. 2002; Langmore et al. 2009). Fan-tailed gerygones on the other hand do not reject shining bronze-cuckoo eggs, thus they would observe a cuckoo hatchling in the nest much later after having been exposed to an adult cuckoo parasitizing their nest. It thus seems unlikely that exposure to a cuckoo might lead to lasting anti-parasite responses in fan-tailed gerygones.

Although the mobbing response by fan-tailed gerygones was different toward each model, there was no difference in the vocal structure of the alarm calls across models and thus no evidence of referential meaning specific to a particular threat in contrast to what is observed in other species (Gill and Sealy 2004). During the presentation trials, neighboring conspecifics were rarely attracted by the calls of the nest owners in response to the cuckoo model presentation. In reed warblers *Acrocephalus scirpaceus* alarm calls attract conspecific neighbors spreading the message that a brood parasite is present in the area (Welbergen and Davies 2008), but in fan-tailed gerygone we only rarely observed conspecific neighbors approaching a pair while mobbing a cuckoo model. Rather, alarm calls of the fan-tailed gerygones seem to be mainly aimed at increasing the attention of the breeding partner toward an approaching danger, as the alarm call phrase has a similar structure to the contact calls used by the pair during foraging (authors' unpublished data). Thus, the alarm calls might have a social function, as in the splendid fairy-wren *Malurus cyaneus*, in which naïve family members identify a parasite by observing more experienced conspecifics mobbing the cuckoo (Feeney and Langmore 2013).

In a previous study, we showed that fan-tailed gerygone parents spend long periods away from the nest during laying (Attisano et al. 2019). Although this behavior might decrease nest predation, it allows enough time for the shining bronze-cuckoo to parasitize the nest without detection by the host. In this study we show that fan-tailed gerygones responded to different levels of threat to the nest and to their own survival. Adult gerygones defend their nest from a predatory shining bronze-cuckoo because attacking this small nest predator bears a low risk for the adults' own survival. Thus, they do not seem to have developed a frontline anti-parasite response but rather an anti-predator defense against their brood parasite. Ultimately, because parasitism by an adult cuckoo cannot be avoided, the nestling ejection response is the better option to decrease brood losses due to parasitism. This promoted a refined recognition ability of the alien nestling with very low rates of recognition errors but that is not associated with an awareness of parasitism risk by host parents.

Author contributions

A.A. conceived the study, designed the experiment, conducted fieldwork, analyzed the data and wrote the first draft. K.H. designed the experiment and conducted fieldwork. R.G. conceived the study. J.T. conceived the study and designed the experiment. All authors provided advice to the analyses and edited the article.

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Supplementary material

Supplementary material can be found at <https://academic.oup.com/cz>.

Conflict of interest

We declare that we have no competing interest.

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Article

Discrimination and ejection of eggs and nestlings by the fan-tailed gerygone from New Caledonia

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Abstract

Nestling rejection is a rare type of host defense against brood parasitism compared with egg rejection. Theoretically, host defenses at both egg and nestling stages could be based on similar underlying discrimination mechanisms but, due to the rarity of nestling rejector hosts, few studies have actually tested this hypothesis. We investigated egg and nestling discrimination by the fan-tailed gerygone *Gerygone flavolateralis*, a host that seemingly accepts nonmimetic eggs of its parasite, the shining bronze-cuckoo *Chalcites lucidus*, but ejects mimetic parasite nestlings. We introduced artificial eggs or nestlings and foreign gerygone nestlings in gerygone nests and compared begging calls of parasite and host nestlings. We found that the gerygone ejected artificial eggs only if their size was smaller than the parasite or host eggs. Ejection of artificial nestlings did not depend on whether their color matched that of the brood. The frequency of ejection increased during the course of the breeding season mirroring the increase in ejection frequency of parasite nestlings by the host. Cross-fostered gerygone nestlings were frequently ejected when lacking natal down and when introduced in the nest before hatching of the foster brood, but only occasionally when they did not match the color of the foster brood. Begging calls differed significantly between parasite and host nestlings throughout the nestling period. Our results suggest that the fan-tailed gerygone accepts eggs within the size range of gerygone and cuckoo eggs and that nestling discrimination is based on auditory and visual cues other than skin color. This highlights the importance of using a combined approach to study discrimination mechanisms of hosts.

Key words: begging calls, brood parasitism, co-evolutionary arms race, egg discrimination, nestling discrimination, nestling polymorphism.

Rejection of brood parasite eggs is a common host defense against brood parasitism, whereas seemingly only few hosts reject nestlings of the brood parasite (Davies 2015; Soler 2017). Two main models attempted to explain the evolution of these 2 host defense strategies: “strategy blocking” (Britton et al. 2007) and “rarer enemy” (Grim 2006). Both models are extensions of the “rare enemy effect,” which suggests that it is not advantageous to develop a defense against a

rare enemy because adaptations are costly (Dawkins 1982). Therefore, high rates of egg rejection make the parasite nestling a rare enemy, which could decrease the selection pressure to evolve host defenses at the nestling stage. However, the 2 models have different predictions. Following the “strategy-blocking” model, a strategy that would be adaptive on its own can be “blocked” by another strategy with lower costs and higher fitness pay-offs (Britton et al.

2007). Thus, mixed strategies of egg and chick rejection should not co-exist in a host population (Britton et al. 2007). In the “rarer enemy” model, any factor preventing parasitism at the nestling stage would also prevent selection for parasite nestling recognition because the host encounters parasite nestlings at a lower frequency than parasite eggs (Grim 2006). This model predicts that nestling discrimination should evolve in hosts that are forced to accept the parasite egg for any reason but also that imperfect egg and nestling discrimination can co-exist in the same host (Grim 2017). The latter scenario seems to be supported by a few hosts that reject (by nest abandonment or ejection) the parasite nestling but usually accept the parasite egg even if it is highly dissimilar from the host eggs (Langmore et al. 2009a; Gloag et al. 2014). However, there is a scarcity of studies testing if and how the 2 strategies can co-exist in the same host and which discrimination cues are employed in one or the other. This might rather be a consequence of a much larger research effort devoted to the laying and incubation stages compared with the nestling stage than of the rarity of nestling rejector hosts (Grim 2007, 2017).

The discrimination of parasite nestlings can involve the use of context-specific cues such as begging calls (Langmore et al. 2003, 2008; Anderson et al. 2010), which are clearly of no help for the discrimination of the parasite egg. The behavior of the parasite nestling could potentially contribute to discrimination; however, the evidence suggests begging calls might act as a super-stimulus to elicit parental feeding but is not necessarily used by the host as a cue for discrimination (e.g., wing shaking; Tanaka et al. 2011). Other cues, for example, odor or body size of the parasite nestling, have not been studied in detail (Grim 2017). On the other hand, visual cues such as color and luminance might potentially be used for the discrimination of both parasite eggs and nestlings. For example, many hosts discriminate the parasite eggs based on markings (Moskát et al. 2008; Spottiswoode and Stevens 2010; Caves et al. 2015) and coloration (Yang et al. 2016; Liang et al. 2017). Similarly, hosts can use natal down and plumage coloration of the parasite nestling as discrimination cues (De Mársico et al. 2012; Noh et al. 2018). Therefore, if a host uses cues that potentially allow discriminating both eggs and nestlings of the parasite, for example, visual cues such as color and luminance, then the 2 rejection strategies might co-exist in a host population, which would support the predictions of the “rarer enemy” model scenario. On the other hand, if a host relies on cues that are only effective at one stage, for example begging calls that could allow discriminating the parasite nestlings but not eggs, then the 2 rejection strategies cannot co-exist in the host population which would support the predictions of the “strategy blocking” model scenario.

In this study, we investigate egg and nestling discrimination in the fan-tailed gerygone *Gerygone flavolateralis*, which is the exclusive host of the shining bronze-cuckoo *Chalcites lucidus* in New Caledonia. The gerygone ejects newly hatched cuckoo nestlings from the nest (Sato et al. 2015; Attisano et al. 2018), but seemingly always accepts the cuckoo egg. The shining bronze-cuckoo egg has a dark olive-brown color and is larger in size than the gerygone egg. Thus, the cuckoo egg might either escape host discrimination if it was cryptic in a dark dome-shaped nest (Langmore et al. 2009a) or evade ejection if the host was unable to grasp the parasite egg in its bill (Moksnes et al. 1991; Rasmussen et al. 2010). The physical limitations of a host to grasp and eject the parasite egg have been assessed using 2 indices of host bill size: the tomial ratio (Rothstein 1975), which is the ratio of the bill length of the host and the width of the parasite egg, and the grasp-index (Rohwer and Spaw 1988),

which is the product of bill length and bill width. Both indices have been used to compare ejection rates in hosts of the brown-headed cowbird *Molothrus ater* (Rasmussen et al. 2010) and common cuckoo *Cuculus canorus* (Moksnes et al. 1991); however, a similar comparative analysis for hosts of bronze-cuckoos within the Australasian region is still lacking.

The New Caledonian cuckoo-gerygone system also includes nestling polymorphism (Sato et al. 2015) as the nestlings of both the host and the parasite have 2 skin color morphs, pinkish-gray (bright) and dark-gray (dark). The 2 host nestling morphs can co-exist in mixed broods and the cuckoo morphs mimic several visual features of the host morphs such as presence of natal down and coloration of gape flanges and skin (Attisano et al. 2018). Polymorphism in egg appearance is known to occur in several parasite–host systems (Gibbs et al. 2000; Yang et al. 2020); however, nestling polymorphism is a much rarer occurrence in birds (Kilner 2006) and the New Caledonian system is, to our knowledge, the only example of nestling polymorphism in both the host and parasite. This system thus offers the unique possibility of investigating egg and nestling ejection strategies in the same host by comparing cues that are potentially shared between strategies (e.g., visual cues such as color and luminance) and cues that are specific to only one stage (e.g., egg size or nestling begging calls).

We conducted a series of experiments using artificial eggs or nestlings and cross-fostered host nestlings to test specific cues involved in the discrimination of eggs and nestlings (Table 1). We used artificial eggs varying in their appearance and size to test if fan-tailed gerygones discriminate eggs based on visual cues and if ejection is constrained by the egg size. Similarly, we used artificial nestlings mimicking the 2 nestling morphs to test if nestling ejection is based on visual cues such as skin color. We additionally cross-fostered fan-tailed gerygone nestlings to test the relative importance of skin coloration (same or different than the foster brood), timing (before or after the foster brood), and natal down (present or absent) in the ejection response of the host. Finally, we compared begging calls of parasite and host nestlings to assess if fan-tailed gerygones might use auditory cues for nestling discrimination. We hypothesized that: (1) fan-tailed gerygones are able to discriminate artificial eggs based on their appearance but do not eject large eggs, thus the cuckoo egg is not cryptic in the gerygone nest but evades ejection because of its size; (2) fan-tailed gerygones discriminate foreign nestlings based on their skin color and natal down, thus they should more often eject artificial nestlings and foreign cross-fostered nestlings if these do not match the appearance of the brood; (3) fan-tailed gerygones additionally use auditory cues to discriminate parasite nestlings from their own chicks.

Materials and Methods

Fieldwork and model species

We conducted fieldwork at 3 sites on the main island (Grande Terre) of New Caledonia during 8 breeding seasons (September–January) in 2011/12–2015/16 and 2017/18–2019/20: Parc des Grandes Fougères (PGF, 21°37' S, 165°45' E), Farino (21°39' S, 165°46' E) and Domaine de Deva (Deva, 21°35' S, 165°22' E). The field sites include areas of tropical rainforest, thicket, and savannah. The fan-tailed gerygone is a small insectivorous bird (adult mass 6.0–6.2 g, length 10 cm) and breeds from September to January with a peak in October/November (Attisano et al. 2019). We searched for active nests in known territories and by following adults flying to their nests. The dome-shaped nests can be located at various

Table 1 Cues for ejection of foreign eggs and nestlings tested in each experiment (indicated by the symbol "X")

	Color	Luminance	Color-match of the brood	Size	Natal down	Timing of introduction
Artificial eggs Exp. 1	X			X		
Artificial eggs Exp. 2		X		X		
Artificial nestlings			X			
Cross-fostered nestlings			X		X	X

heights (range 0.4–20 m), but for practical reasons, we restricted our study to nests lower than 3 m. We found a total of 344 active nests (containing at least one host or parasite egg/nestling), out of which 68 were parasitized and 74 (72 non-parasitized and 2 parasitized) survived to fledging. Upon finding an active nest, we determined the age either of the eggs by candling (Lokemoen and Koford 1996) or of the nestlings based on their development stage. This allowed us to estimate the nest age considering a 2-day laying interval between eggs, 18 days of incubation, and 14 days of brooding (Attisano et al. 2019). Despite extensive video recording (>16,000 h during laying and incubation), we never observed gerygones removing foreign material (leaves, twigs, berries, or similar) from the nest and never directly recorded the presence of such material in the nest, because the dome-shaped structure reduces the chances of foreign material falling inside the nest. We also never observed gerygones removing unhatched eggs, although they remove eggshell soon after hatching of the chicks (gerygone or cuckoo). We observed 4 cases in which a nestling (6–9 days old) died of natural causes, 2 in a single-chick brood, and 2 in a 2-chick brood, but the parents removed none of the carcasses. Three of these nests were soon abandoned by the parents (2 single-chick broods and one 2-chick brood), whereas at one nest (with a 2-chick brood) the parents raised the surviving nestling until fledging without removing the dead chick.

The size of an average fan-tailed gerygone egg is 18.4×13.3 mm (range: length 15.6–21.0 mm, width 12.0–14.6 mm) with a mass of 1.3 ± 0.02 g (mean \pm 95% confidence interval [CI], $n = 122$), whereas an average shining bronze-cuckoo egg is 22.5×14.8 mm (range: length 18.9–23.8 mm, width 13.0–15.8 mm) with a mass of 1.9 ± 0.04 g ($n = 22$). There is no egg mimicry as the cuckoo egg is covered with a dark-brown pigment and is clearly distinct from the whitish-gray egg with brown speckles of its gerygone host (Figure 1A). We never observed ejection of own or cuckoo eggs by the fan-tailed gerygone, neither directly on camera nor indirectly via a reduction in clutch size during the incubation period. We also have no reason to believe that fan-tailed gerygones reject cuckoo eggs as they rarely abandoned their nest (6% of 344 active nests) and the presence of a cuckoo egg in the nest did not increase the frequency of nest abandonment ($\chi^2 = 1.299$, $df = 1$, $P = 0.254$). The cuckoo always removes one host egg before laying its own and multiple parasitisms of the same nest are rare (1 out of 68 parasitized nests). As part of other concurrent field observations, we temporarily swapped real gerygone or cuckoo eggs with a model egg mimicking their respective size and coloration in >40 occasions. We placed the real eggs in incubators for a period of 1–7 days and returned the hatchlings to their nests. We never observed the fan-tailed gerygone ejecting artificial eggs or abandoning the nest.

Hatchlings of the host and parasite have similar size, but cuckoo hatchlings are slightly heavier (1.4 g, range 1.2–1.8, $n = 3$) than fan-tailed gerygone chicks (1.1 g, range: 0.9–1.5; $n = 46$). Out of 222 host chicks, 75% were bright and 25% dark, and out of 130 host broods, 69% contained only bright chicks, 23% only dark chicks

and 8% were mixed. The dark morph of the parasite was rare as out of 26 parasite chicks all but 1 was bright. The bright parasite morph mimics visual features of both host morphs (Attisano et al. 2018), nevertheless gerygones ejected 88% (29 of 33) of newly hatched cuckoo chicks within few hours from hatching, regardless if the cuckoo chicks did ($n = 11$) or did not match ($n = 4$) the host brood color. All the accepted cuckoo chicks (4 of 33) evicted the still unhatched host eggs.

Artificial eggs and nestlings

We conducted 2 egg ejection experiments to test if fan-tailed gerygones used color, luminance, or size as cues for the discrimination and ejection of foreign eggs (Table 1). We made artificial eggs using modeling clay (Fimo Air, Staedler), which hardened after being exposed to air but still remained soft enough to record imprints of bill marks on the surface, allowing us to record if the host attempted to peck or grasp the artificial egg without being able to remove it from the nest. We prepared all artificial eggs at least 1 week before their use in the experiments and left them to dry in a ventilated location to allow any residual smell left by human manipulation or paint to dissipate. We controlled for the mass of the artificial eggs by inserting fishing beads in the clay to reach the average mass of a gerygone egg (1.3 g).

For Experiment 1, we used 3 types of artificial eggs (Figure 1B): parasite-like (brown, mimicking the parasite egg), host-like (whitish-grey with brown speckles, mimicking the host egg), and blue (novel visual stimulus to the host as no other passerine in New Caledonia lays blue eggs). We hand-rolled the clay into an egg shape and painted it with nontoxic paints (Turner Color Works Ltd, Osaka, Japan). The parasite-like and host-like eggs mimicked as close as possible the color of cuckoo and gerygone eggs, respectively, whereas the color of the blue model was highly dissimilar from both cuckoo and gerygone eggs. The artificial eggs had a standardized size of 10×7 mm, which is about 50% of the length and width of an average gerygone egg. The primary reason for this experiment was to test if gerygones would remove foreign eggs that are much easier to grasp compared with eggs of normal size (Table 1). The second aspect was to test if the frequency of ejection depended on the color of the model. If the cuckoo eggs were cryptic, we would expect a lower ejection rate of parasite-like artificial eggs compared with blue or host-like artificial eggs (Table 1).

For Experiment 2, we used 4 types of artificial eggs differing in luminance and size (Figure 1C): high luminance combined with large size, high luminance and medium size, low luminance and large size, low luminance, and medium size. We chose the size of these artificial eggs to match the size range of real eggs encountered by gerygones in their nest. Thus, the large artificial eggs matched the size of a cuckoo egg (22×14 mm), which is the largest egg possible in this system, and the medium-sized artificial eggs matched the size of the smallest gerygone egg that we measured in the field (15×11 mm). We molded the clay into an egg shape using a custom-made plastic

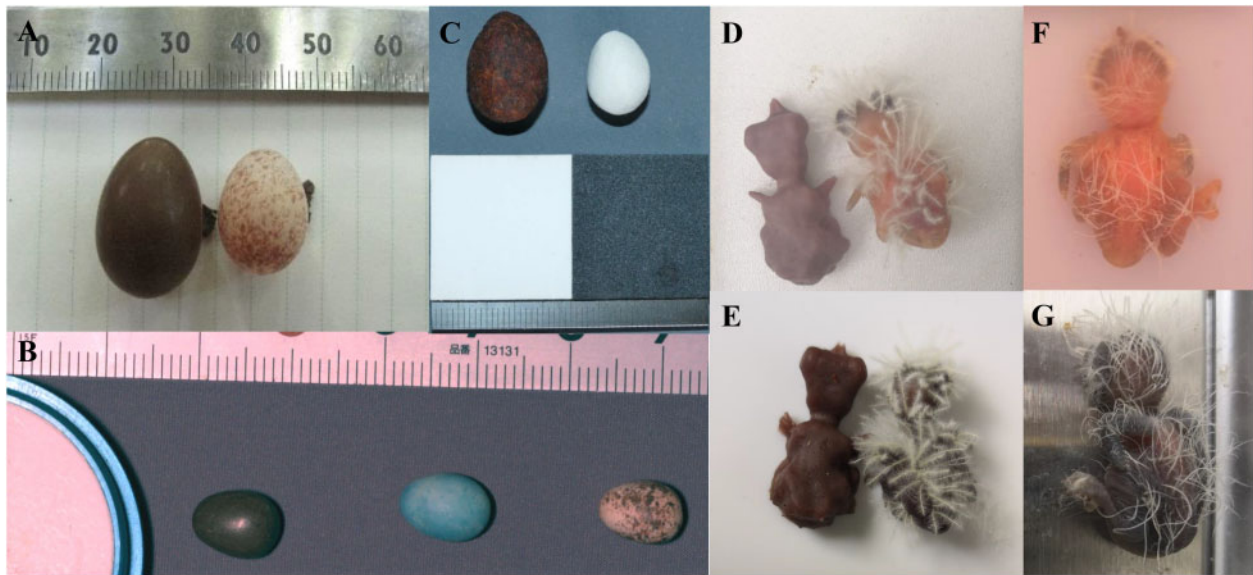


Figure 1. (A) Eggs of shining bronze-cuckoo (left) and fan-tailed gerygone (right). (B) Artificial eggs for Experiment 1: parasite-like brown (left), blue (center), and host-like whitish-gray with brown speckles (right). (C) Artificial eggs for Experiment 2: low reflectance (left, only large size shown) and high reflectance (right, only small size shown). (D) Bright artificial nestling (left) with bright fan-tailed gerygone nestling (right). (E) Dark artificial nestling (left) with dark fan-tailed gerygone nestling (right). (F) Bright shining bronze-cuckoo nestling. (G) Dark shining bronze-cuckoo nestling. All nestlings in the photos are newly hatched (Day 0). Gerygone and cuckoo nestlings are not at the same scale.

mold to achieve artificial eggs of consistent size. We painted the low luminance models with 2 layers of nontoxic paint (Copic Ciao Marker E57, walnut brown) to mimic as closely as possible the luminance of a dark-brown shining bronze-cuckoo egg, whereas the high luminance models kept the original white color of the modeling clay to achieve higher luminance than both gerygone and cuckoo eggs. The first aim of this experiment was to test if the gerygones ejected artificial eggs within the size range of real eggs (Table 1). The second aim was to test if the frequency of ejection depended on the luminance of the model. If the low luminance model was cryptic then we would expect it to be less often ejected than the high luminance model irrespective of the size (Table 1).

We then investigated if fan-tailed gerygone parents ejected foreign nestlings that did not match the color of their own brood (Table 1). We used artificial nestlings made out of soft (to mimic the skin of newly hatched nestlings) silicone rubber (Ecoflex 00-30, Smooth-on), casted in a custom-made mold allowing us to produce models of standardized size (27×12 mm) and mass (1.2 g) similar to newly hatched gerygone and cuckoo nestlings. We painted the artificial nestlings with non-toxic silicone paint (Silk-pig, Smooth-on) to obtain 2 types, bright and dark, which mimicked as closely as possible the 2 morphs of both gerygone and cuckoo nestlings (Figure 1, D–G).

We measured color and luminance of the artificial eggs and nestlings using multispectral images taken with a Fuji IS-Pro full-spectrum digital photo camera. We used the MICA Image Analysis Toolbox plugin (Troscianko and Stevens 2015) for ImageJ (Schneider et al. 2012) to convert the multispectral images to cone catch using the peacock violet sensitive (VS) visual model (Hart 2002) as both the parasite and host in New Caledonia are predicted to have a VS visual system (Aidala et al. 2012a, 2012b). We verified that the artificial eggs mimicked as closely as possible the color (Experiment 1) and luminance (Experiment 2) of cuckoo and gerygone eggs and that artificial nestlings (Experiment 3) mimicked as

closely as possible the color and luminance of the respective cuckoo and gerygone nestling morphs (Supplementary materials S1 and S2).

Ejection of artificial eggs

We introduced artificial eggs in active non-parasitized nests at laying or early incubation (<5 days after first egg laying) stages that we randomly assigned to a treatment. On Day 1, we randomly chose one host egg from the nest, temporarily placed it in an incubator, and replaced it with the first artificial egg of the treatment. For Experiment 1, the treatment was one of the 6 possible presentation sequences (consecutive combinations of the 3 artificial egg colors). The complete egg presentation lasted for 6 days during which we introduced at 2-day intervals the 3 (either blue, parasite-like, or host-like) artificial egg types to the nest. We used 15 nests at 2 sites (PGF, Farino) for a total of 44 model presentations (at one nest we could not introduce the host-like egg because of depredation). For Experiment 2, we assigned each nest to a size treatment (either large or medium) and introduced the 2 egg types (a low-luminance and a high-luminance type) at 2-day intervals in the nest. We randomized the presentation order for each nest. We used 14 nests at all 3 sites for a total of 25 egg presentations (at 3 nests we could not introduce the second artificial egg in the sequence because of depredation or adverse weather conditions).

We confirmed acceptance or ejection of the artificial egg at each nest check and replaced it (if still present) with the next one in the treatment sequence. We checked for the presence of bill marks or scratches on the surface of accepted models to monitor unsuccessful ejection attempts (failed puncture- or grasp-ejection) by the host. On the final day of the experiment, we removed the last artificial egg in the sequence (if still in the nest) and returned the host egg from the incubator to the nest. In case the original nest was meanwhile depredated, we introduced the egg to the next available gerygone nest that was not used for the experiments and in a similar developmental stage.

Ejection of artificial nestlings

We introduced artificial nestlings into 30 nests (23 nonparasitized and 7 parasitized) at all 3 sites. We randomized the color of the artificial nestling introduced into the nest, thus resulting in 2 experimental combinations: model matching the host brood color (match of the whole host brood in monomorphic broods or of at least one host nestling in mixed broods, $n = 19$) and model not matching the host brood color ($n = 11$). We introduced the artificial nestlings into the nest after at least one host nestling had hatched (range 0–6 days of age), so that we knew the skin color of the first hatchling. We checked all nests 2–4 days after the introduction of the artificial nestling to determine the complete host brood color composition, to confirm the acceptance or ejection of the model, and to remove accepted artificial nestlings from the nest. As we were not able to keep nestlings in captivity for the duration of the presentation, we did not replace one of the host nestlings with the models, thus artificial and host nestlings co-existed in the nest.

Ejection of cross-fostered gerygone nestlings

We occasionally had gerygone nestlings hatching in incubators that we could not reintroduce into their original nests, because these were meanwhile lost to depredation. As hand-raising was not possible, we introduced these hatchlings into other available gerygone nests, where they had at least a chance of survival. This allowed us to conduct quasi-experimental observations using cross-fostering.

We conducted observations at 14 nonparasitized nests at 2 sites (PGF, Farino). The nests were between 4 days before to 4 days after hatching and contained at least one unhatched host egg (infertile eggs or undeveloped embryos), thus we could replace this egg with the cross-fostered nestling and avoid an increase in brood size. We introduced the nestlings randomly in respect to the foster brood color, thus the cross-fostered nestlings were either matching ($n = 11$) or not matching ($n = 3$) the foster brood color, and in relation to the time of hatching of the foster brood, thus the cross-fostered nestlings were introduced either before hatching of the foster brood ($n = 7$) or after at least one of the foster host nestlings had hatched ($n = 7$). Cuckoo nestlings have a sparser and less conspicuous natal down than gerygone nestlings (Figure 1, D–G), thus host parents might use this as a visual cue for the discrimination of the parasite. Therefore, we manipulated the appearance of some cross-fostered nestlings by trimming their natal down with fine forceps so that the introduced nestlings either had ($n = 8$) or lacked ($n = 6$) down feathers to verify if this increased or decreased chances of acceptance. We checked each nest after 2 days to confirm acceptance or ejection of the cross-fostered nestling.

Tomial ratio and grasp index

We caught adult fan-tailed gerygones using mist nets and measured bill length as the distance from the commissural point to the tip of the upper mandible and bill width as the distance between the commissural points. We compared the calculated tomial ratio and grasp index with respective measurements obtained from common hosts of the brown-headed cowbird (Rasmussen et al. 2010) and common cuckoo (Moksnes et al. 1991) to understand if bill size of the fan-tailed gerygone might constrain egg ejection.

Begging calls

We recorded begging calls of cuckoo and gerygone nestlings from the day of hatching (Day 0) until Day 13 of 27 nests at all 3 sites at about 2-day intervals between recording sessions for each given nest

(depending on weather conditions). We inserted a Shure SM93 micro condenser microphone into the external bottom layer of the nest at a distance of 1–2 cm from the floor of the incubation chamber thus allowing us to record the faint begging calls of newly hatched chicks with a Tascam DR-40 digital recorder. From the recordings, it was possible to determine when the parents landed on the nest (marked by a loud thump) and called their chicks causing a begging response from the nestlings. Starting from this point, we extracted 5-s long audio tracks of the begging calls and visualized them in a spectrogram (Hann window, 3 dB bandwidth 135 Hz, 90% overlap, Hop size 51 samples, DFT 512 samples, Grid spacing 93.8 Hz). We defined a call as a single uninterrupted trace on the spectrogram. We measured the number of calls produced by the nestlings within the 5-s period, the time interval between calls, call duration, lowest frequency, highest frequency, frequency bandwidth, peak frequency (frequency at which the highest amplitude occurs), and call entropy (amount of disorder in the call, with 0 being a call of constant frequency) in Raven Pro version 1.6 (Center for Conservation Bioacoustics 2019). We used the nest as the sample unit and averaged the measurements for each parameter across the recordings at each nest. Some of the nests contained several fan-tailed gerygone nestlings during the recording sessions (as the brood size can range from 1 to 3 chicks), and in these cases, we measured all the begging calls in the recordings and obtained an average value for each call parameter. We recorded cuckoo nestlings ($n = 7$) only when they were the only chick in the nest, that is, before any of the host eggs hatched or when reared alone in the nest.

Nest illumination

We measured illumination within the incubation chamber of 68 nests (of which 21% were parasitized) at all 3 sites using a Sanwa LX2 illuminance meter. We collected measurements between 800 and 1,600 h during sunny days with no cloud cover. For each nest, we took 3 measurements by placing the meter's sensor inside the incubation chamber and averaged the measurements for each nest. We conducted egg ejection tests (with artificial eggs from Experiment 2) at 8 and nestling ejection tests (with artificial nestlings from Experiment 3) at 14 of the 68 measured nests.

Stats

We conducted the experiments over multiple breeding seasons, but we found that year was not an influential factor and thus removed it from the analyses. We then investigated which variables contributed to the ejection of artificial eggs, artificial nestlings, and cross-fostered nestlings by building models in which the response variable was the ejection of the focal egg or nestling (0 = accepted, 1 ejected). For the egg ejection experiments, we used generalised linear mixed models (GLMMs) that included type of artificial egg, site (to account for a population effect on ejection), and day of the season (range 1–130, 1 = 9th September, to account for a temporal effect on ejection) as fixed effects and nest ID as a random effect (to account for multiple egg presentations at the same nest). We tested for the effect of egg size on ejection with a generalised linear model (GLM) that included size of all experimental eggs (small, medium, and large) as a fixed effect. For ejection of the artificial nestlings, we used a GLM that included color-match of the artificial and the host nestlings (0 = no match, 1 = match), parasitism (0 = nonparasitized, 1 = parasitized), site and day of the season as fixed effects. For the ejection of cross-fostered nestlings, we used a GLM that included color-match of the cross fostered nestling with the foster brood

(0 = no match, 1 = match), presence of down feathers (0 = present, 1 = lacking), and timing of introduction into the nest (before hatching of host nestlings, after hatching of host nestlings) as fixed effects. In addition, we investigated the influence of nest illumination on ejection of cuckoo and artificial nestlings using 2 GLMs that included ejection of either the cuckoo or artificial nestling as a binomial response variable and average nest illumination as a fixed effect.

Fan-tailed gerygone host parents usually eject the cuckoo nestling within the first 24–48 h after hatching and we never observed ejection or nest abandonment by host parents later than 4 days after hatching (Attisano et al. 2019). We thus divided the recordings of begging calls into 3 age groups based on chick development, begging call structure, and probability of ejection of the parasite chick: 0–3 days (early nestling period, hatchlings and young chicks, quiet begging calls with simple structure, very high probability of ejection of the parasite), 4–7 days (mid nestling period, begging calls beginning to present a defined structure, low probability of ejection of the parasite), 8–13 days (late nestling period, loud begging calls with a complete defined structure, no ejection, and no abandonment of the nest). We obtained an average value for each nest in each developmental group using multiple calls from the same nest (range 1–4 recordings) and used these values in a discriminant analysis. We first tested if a discriminant model could differentiate among host, accepted cuckoo, and ejected cuckoo nestlings based on the measurements collected during 0–3 days from hatching. We then used the begging call measurements collected during the early, mid, and late nestling periods to check if the discriminant model was able to differentiate between species according to the developmental stages of the chicks (i.e., if the cuckoo nestlings mimicked the hosts at any stage of the nestling period). Finally, we built mixed models to test for the effect of species and nestling age on the 8 measured call parameters using the full dataset of the recordings of begging calls collected from Days 0 to 13 from hatching (i.e., the average value from all the recording sessions from each nest within each day). We checked if begging call parameters followed a normal distribution and then used each parameter as a response variable in either a LMM or GLMM which included species (cuckoo, gerygone), chick age (range 0–13 days), and their interaction as fixed effects and nest ID as random effect to account for repeated measures on the same nest.

We built GLMMs in R version 3.6.3 (R Core Team 2019) using the package lme4 (Bates et al. 2015) and extracted *P*-values for the variables in each model using the function ANOVA from the package car (Fox and Weisberg 2019). We used additional nonparametric tests when variables did not meet the assumption of a normal distribution and report averages with 95% CIs.

Results

Ejection of artificial eggs

Fan-tailed gerygone ejected only the small artificial eggs from Experiment 1 (10 × 7 mm), but accepted all medium and large artificial eggs from Experiment 2 (15 × 11 mm and 22 × 14 mm). In Experiment 1, gerygones ejected 73% of the blue eggs compared with 53% of the parasite-like and 43% of the host-like eggs (Figure 2A) and we confirmed by video recording that they removed eggs by grasp-ejection (Supplementary material S4). For this egg ejection experiment, we found that neither egg type (GLMM; $\chi^2 = 2.562$, $df = 2$, $P = 0.278$), day of the season (GLMM; $\chi^2 = 2.525$, $df = 1$, $P = 0.112$) nor site (GLMM; $\chi^2 = 3.762$, $df = 1$, $P = 0.052$) had an

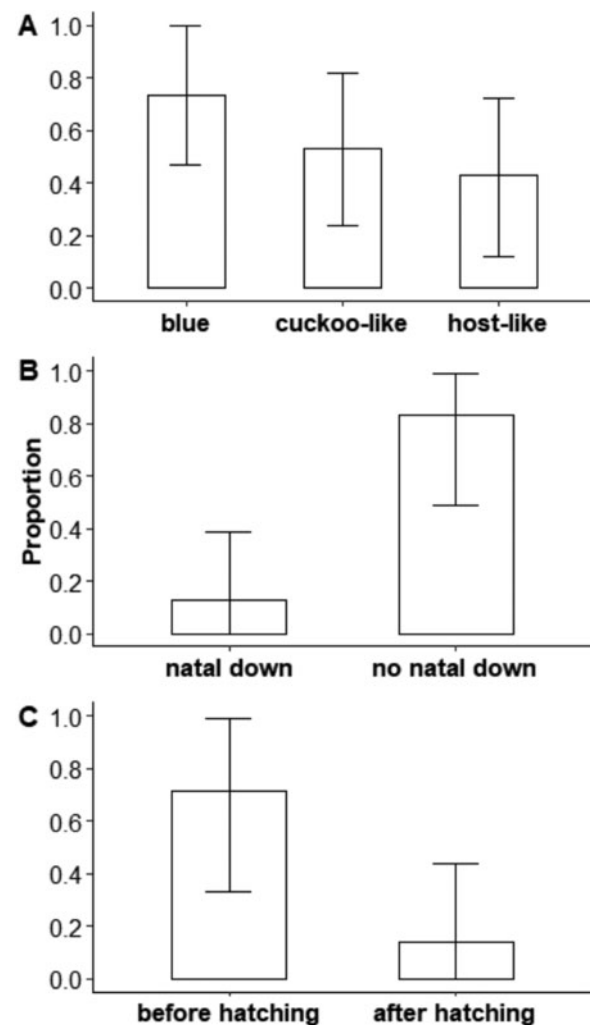


Figure 2. Proportion (with 95% Bonferroni CIs) of ejected artificial eggs in Experiment 1 (A, $n = 15$ blue, $n = 15$ parasite-like, $n = 14$ host-like) and ejected cross-fostered nestlings according to presence/absence of natal down (B, $n = 8$ natal down present, $n = 6$ natal down absent) and timing of introduction to the foster brood (C, $n = 7$ before hatching of the foster brood, $n = 7$ after hatching).

effect on the ejection of the egg models. The presentation sequence of the egg models also had no influence on ejection rates ($\chi^2 = 4.374$, $df = 5$, $P = 0.497$). The size of the artificial eggs was the only factor determining the ejection response, with small eggs often ejected but medium and large eggs always accepted (GLM; $\chi^2 = 32.806$, $df = 2$, $P < 0.001$). All the accepted eggs showed no presence of scratches or marks, suggesting that the host did not attempt to pierce or grasp them.

Ejection of artificial nestlings

Fan-tailed gerygones accepted 23 (77%) and ejected 7 (23%) artificial nestlings. For comparison, during 8 field seasons, gerygones accepted 4 (12%), and ejected 29 (88%) cuckoo hatchlings. At the 8 parasitized experimental nests gerygones ejected 1 and accepted 7 artificial nestlings, whereas gerygones ejected the cuckoo nestlings in 6 occasions within 1–2 days from hatching (in 2 nests the cuckoo egg did not hatch). The ejection of the artificial nestling was not influenced by color-matching of the host brood (GLM; $\chi^2 = 0.303$,

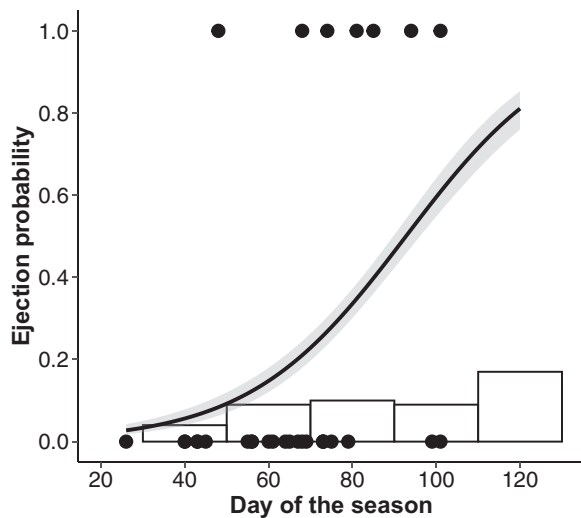


Figure 3. Logistic regression (estimate = 0.053, $SE = 0.028$) for the probability of fan-tailed gerygones to accept (0) or eject (1) the artificial nestling in relation to day of the season (1 = 9th September). Black points are artificial nestling ejection trials and the gray area around the regression line is the 95% confidence band. Vertical bars are the proportions of cuckoo nestlings ejected by host parents observed within 20-day periods during the 8 breeding seasons.

$df = 1$, $P = 0.582$), site (GLM; $\chi^2 = 3.405$, $df = 2$, $P = 0.182$), or the nest being parasitized (GLM; $\chi^2 = 1.119$, $df = 1$, $P = 0.290$). The probability of ejection of artificial nestlings increased as the season progressed (GLM; $\chi^2 = 6.119$, $df = 1$, $P = 0.013$) along with the proportion of nests with cuckoo nestling ejections (Figure 3).

Ejection of cross-fostered gerygone nestlings

Fan-tailed gerygone foster parents accepted 8 (57%) and ejected 6 (43%) cross-fostered gerygone nestlings. The ejection response was best explained by the lack of down (GLM; $\chi^2 = 11.345$, $df = 1$, $P = 0.001$; Figure 2B) and timing of introduction of the cross-fostered nestling to the foster brood (GLM; $\chi^2 = 6.453$, $df = 1$, $P = 0.011$; Figure 2C), whereas there was no influence of color-match of the foster brood (GLM; $\chi^2 = 3.819$, $df = 1$, $P = 0.051$). We also found no influence of day of the season (logistic regression; $\chi^2 = 2.598$, $df = 1$, $P = 0.107$) nor site ($\chi^2 = 1.027$, $df = 1$, $P = 0.311$) on the ejection of cross-fostered nestlings.

Tomial ratio and grasp index

Bills of adult fan-tailed gerygones ($n = 71$) were on average 12.7 ± 0.1 mm long and 4.0 ± 0.03 mm wide. The average fan-tailed gerygone had a tomial ratio of 0.88 ± 0.02 and a grasp index of 51.2 ± 1.5 mm².

Begging calls

The discriminant model was able to differentiate among gerygone nestlings, accepted cuckoo nestlings and ejected cuckoo nestlings that were no more than 3 days old (Figure 4). The model correctly identified the species of the nestling (as belonging to cuckoo or gerygone) in 91% of the cases ($n = 22$, 1 accepted cuckoo misidentified as a gerygone and 1 gerygone misidentified as an ejected cuckoo) when the nestlings were between 0 and 3 days old, 86% of the cases ($n = 14$, 1 cuckoo misidentified as a gerygone and 1 gerygone misidentified as a cuckoo) when the nestlings were 4 to 7 days old and 83% of the cases ($n = 6$, 1 cuckoo misidentified as a gerygone) when

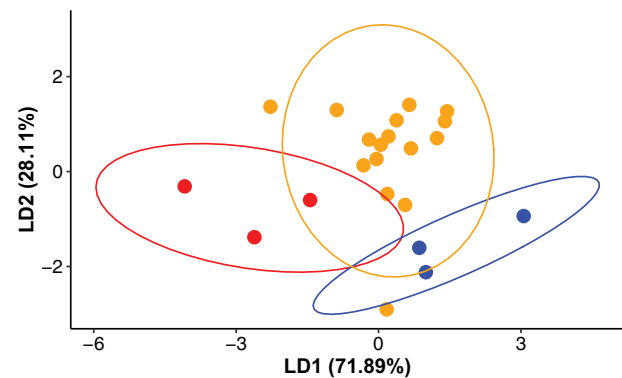


Figure 4. Canonical plot of the first and second linear discriminant functions for the 8 measured parameters of the begging calls in 0–3 days old nestlings. Orange dots are fan-tailed gerygone nestlings, red dots are shining bronze-cuckoo nestlings accepted by host parents and blue dots are shining bronze-cuckoo nestlings ejected by their host parents within 1–2 days from hatching. Ellipses are 95% CIs around the centroid of the distribution for each nesting type.

the nestlings were 8 to 13 days old. The species were defined by significant differences in lowest, highest, and peak frequencies of the begging calls, whereas all begging parameters, with the exception of entropy, varied with the nestling age (Table 2; Figure 5; Supplementary material S3).

Nest illumination

Nests of fan-tailed gerygone had a mean illuminance of 656 ± 170 lux (range 11–3,365 lux). Nests from which gerygones ejected cuckoo nestlings ($n = 9$) had similar illuminance to non-parasitized nests ($n = 47$; GLM, $\chi^2 = 0.013$, $df = 1$, $P = 0.909$). Similarly, nests from which artificial nestlings were ejected ($n = 2$) had similar illuminance to nests in which the artificial nestlings were accepted ($n = 12$; GLM, $\chi^2 = 0.707$, $df = 1$, $P = 0.4$). Nest illumination was neither affected by site (Kruskal–Wallis $\chi^2 = 2.134$, $df = 2$, $P = 0.344$) nor part of the day (morning versus afternoon; Kruskal–Wallis $\chi^2 = 0.705$, $df = 1$, $P = 0.401$).

Discussion

Our results showed that the fan-tailed gerygone does not eject eggs based on their appearance. The gerygones only removed artificial eggs that were small enough to be grasped irrespective of their color, whereas they always accepted artificial eggs matching the size range of gerygone and cuckoo eggs irrespective of their luminance. Thus, egg size, rather than its appearance, is likely to constrain egg ejection in this host. Similarly, the ejection of foreign nestlings (gerygone or cuckoo) is not based on cues such as nestling skin color, but is rather based on other visual cues such as the presence of natal down as well as the timing of introduction. Begging calls might also be used as an additional cue for the discrimination of foreign nestlings. Therefore, fan-tailed gerygones always accept eggs within a natural size range and discriminate eggs and nestlings by using different cues.

We found that the fan-tailed gerygone did also not reject foreign eggs by deserting parasitized clutches as other hosts do (Langmore et al. 2003; Medina and Langmore, 2016). Instead, nest abandonment in the fan-tailed gerygone is rare and is not linked to the presence of a cuckoo egg in the nest. The reason might be that female gerygones invest large resources into egg production (over 20% of the female body mass per egg) and that a pair completes a breeding

Table 2 Average (with 95% CI) parameters of begging calls for cuckoo nestlings accepted by the host parents ($n=3$), cuckoo nestlings ejected by the host parents ($n=3$), and gerygone nestlings ($n=16$) with P -values of mixed models of the difference in begging call parameters between species (gerygone, cuckoo), age of the chick (0–13) and their interaction. Statistically significant results at $P < 0.05$ are in bold

	Cuckoo accepted	Cuckoo ejected	Gerygone	Species	Age	Species*Age
Number of calls in 5 s	4.33 ± 5.02	4.83 ± 8.98	6.93 ± 1.66	0.168	< 0.001	0.218
Interval between calls (s)	1.19 ± 2.54	0.67 ± 1.17	0.75 ± 0.20	0.682	0.005	0.931
Call duration (s)	0.03 ± 0.03	0.07 ± 0.07	0.05 ± 0.01	0.763	< 0.001	0.162
Lowest frequency (kHz)	5.38 ± 1.72	5.19 ± 1.02	6.05 ± 0.35	0.005	< 0.001	0.831
Highest frequency (kHz)	6.41 ± 2.41	6.57 ± 1.15	7.41 ± 0.53	0.005	< 0.001	0.046
Peak frequency (kHz)	6.06 ± 2.33	6.06 ± 1.04	6.96 ± 0.47	0.011	< 0.001	0.270
Frequency bandwidth (kHz)	1.03 ± 0.75	1.38 ± 0.62	1.36 ± 0.31	0.096	< 0.001	< 0.001
Entropy	2.86 ± 1.38	2.77 ± 0.66	2.89 ± 0.38	0.322	0.753	0.043

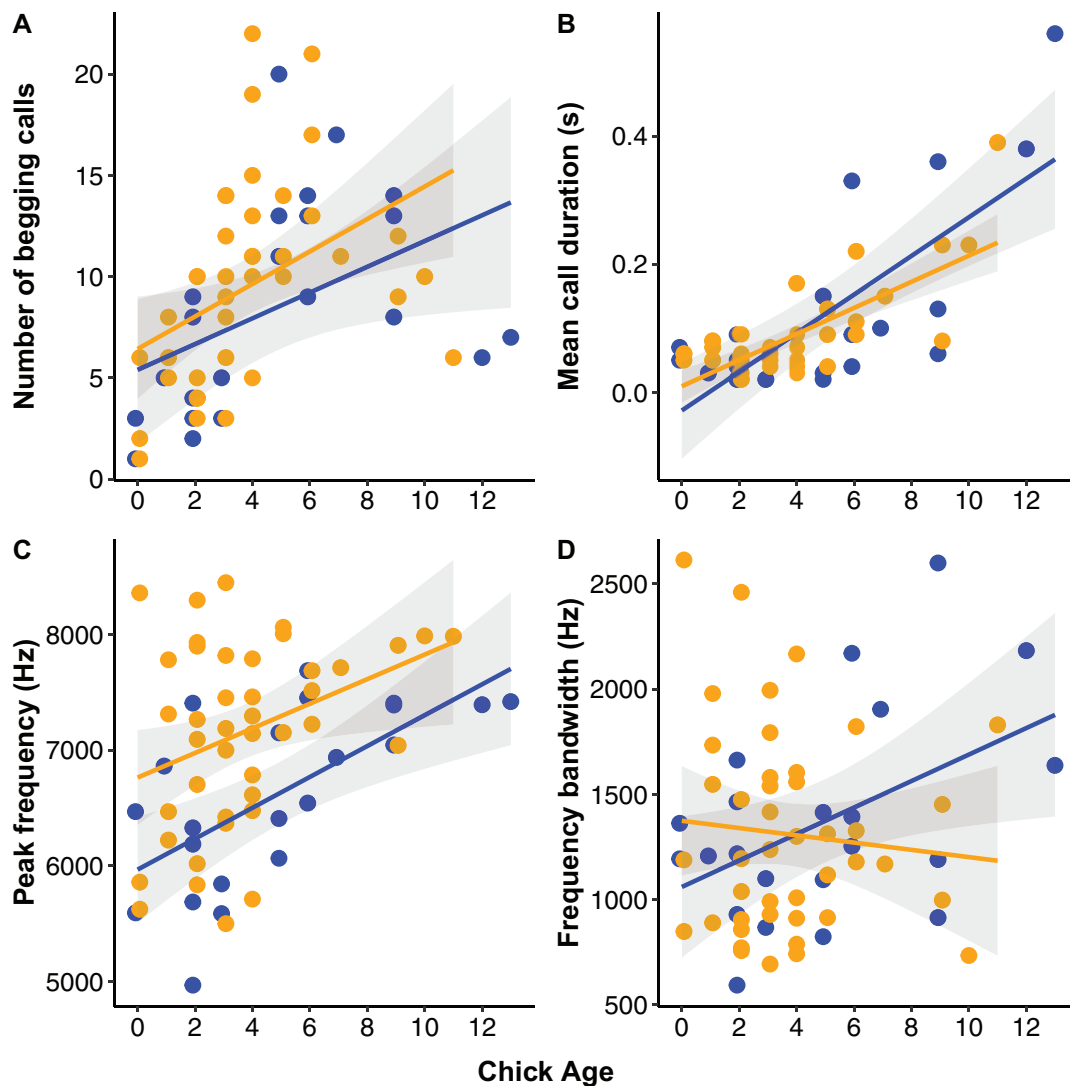


Figure 5. Variation of begging call parameters in relation to age of fan-tailed gerygone (orange) and shining bronze-cuckoo (blue) nestlings. The grey shaded areas around the regression lines are the 95% confidence bands.

cycle in about 10–12 weeks (including nest building, laying, incubation, brooding, and raising the fledglings) which is about half the length of the breeding season. Therefore, nest abandonment might reduce the chances of a successful reproduction within the same

breeding season and thus be too costly as a form of egg rejection strategy in this host.

A possible reason why gerygones never ejected artificial eggs that were at least the size of their own eggs might be that they were not

able to puncture–eject the models because these were made of clay. However, we think that this form of egg ejection is unlikely as we never observed any egg removal by this host, neither indirectly via a reduction in clutch size nor directly on camera despite intensive video monitoring. In addition, we never recorded beak marks on any artificial egg. Another reason for the acceptance might be that fan-tailed gerygones produce small clutches of 2 eggs on average (Attisano et al. 2019), thus accepting the cuckoo egg might prevent further reduction of the host clutch by multiple parasitizing cuckoo females (egg dilution effect in Sato et al. 2010). Alternatively, a strategy of removing eggs from the nest might result in ejection errors and lead to a costly reduction of their own clutch (Davies et al. 1996). Similar rejection experiments conducted on the grey gerygone *Gerygone igata* and the yellow-rumped thornbill *Acanthiza chrysorroa*, which are closely related to the fan-tailed gerygone and are also hosts of the shining bronze-cuckoo, confirm that also these hosts are acceptors of naturally sized artificial eggs (Thorogood et al. 2017; Medina and Langmore 2019). However, our result that fan-tailed gerygones frequently ejected small eggs suggests that another reason why fan-tailed gerygones accept natural eggs (of both host and parasite) might be that the eggs are too large to be grasped and removed from the nest. The tomial ratio and grasp index, which are important indicators for grasp–ejector hosts, would indeed place the fan-tailed gerygone in the group of small hosts from Europe and North America that accept foreign eggs (tomial ratio: 0.62–1.14, Rothstein 1975; grasp index: 53.4–285.0, Rohwer and Spaw 1988; Moksnes et al. 1991; Underwood and Sealy 2006). Therefore, the absence of ejection of eggs of natural size in this host is most likely explained by a combination of the high cost of ejecting the eggs and by physical limitations preventing grasp–ejection of a foreign egg.

Although we did not specifically test for nest sanitation behavior using additional non-egg shaped objects, we think it is unlikely that the small artificial eggs might have been removed because they were seen solely as foreign material. First, studies linking nest sanitation and egg rejection consistently showed that hosts respond differently to non-egg shaped and egg shaped models, suggesting that the latter, even when smaller than real eggs, are likely to be regarded as real eggs by the hosts (Guigueno and Sealy 2012; Honza and Cherry 2017). In our case, the artificial eggs resembled the shape of a gerygone egg and the host ejected all types, not just the alien-looking blue one, at relatively high rates. Second, the dome-shaped nest might prevent foreign material to fall into the nest, thus sanitation in the form of removal of foreign nonegg objects in this host is a much rarer behavior than in open cup nesters. Third, egg discrimination by size and shape does occur in some hosts of brood parasites (Marchetti 2000; Langmore et al. 2003; Guigueno et al. 2014; Taylor and Langmore 2020), and the gerygones frequently removed the small artificial eggs but did not remove artificial eggs matching the size of real eggs. It is thus likely that the fan-tailed gerygone was not physically able to remove eggs of medium or large size, but they were able to grasp–eject the small eggs.

Closed nests have generally lower illumination than open nests (Langmore et al. 2005; Avilés et al. 2006); therefore, Langmore et al. (2009a) hypothesized that dark bronze-cuckoo eggs might escape detection because they are cryptic in this type of nest. Our observation that fan-tailed gerygones frequently ejected the parasite-like small artificial eggs would rather suggest that these were not cryptic because the hosts detected the parasite-like eggs as well as they detected the blue and host-like eggs. However, some gerygone nests have relatively low illumination values meaning that light availability might sometimes be a limiting factor. Superb fairy-wren *Malurus*

spendens build similar dome-shaped nests and reject foreign eggs based on their size rather than color, suggesting they might use tactile cues to discriminate foreign eggs (Langmore et al. 2003). Therefore, the relatively similar ejection rates of small artificial eggs of various colors could also be explained by the fan-tailed gerygone using tactile cues to detect artificial eggs.

There are several possible explanations why fan-tailed gerygones do not rely on the skin color of the artificial nestlings and cross-fostered nestlings as a discrimination cue. First, low illumination within the nests might make color cues less effective. Second, extra-pair copulations and partner changes across multiple seasons could cause variation in the brood coloration and increase the chances that the same parent will encounter both host nestling morphs during its lifetime (Bojarska et al., 2018). Third, cuckoo nestlings also occur in 2 morphs (Sato et al. 2015), thus increasing the phenotypic variation of nestlings, which could lead to higher chances of misidentification. Therefore, skin color alone cannot be a reliable cue for the discrimination of the parasite nestling. However, cuckoo nestlings have a sparser natal down than the host and always hatch earlier than the host chicks, which mean that these can be more reliable cues for the discrimination of the parasite nestling than skin color. Similar results were also found in other hosts that reject bronze-cuckoo nestlings using either hatching order or natal down as cues (Langmore et al. 2009a; Noh et al. 2018). In addition, we found that the shining bronze-cuckoo nestlings only imperfectly mimic the host begging calls, thus gerygone host parents might use sound cues for the discrimination, similarly to other hosts that discriminate the parasite nestlings via their begging calls (Langmore et al. 2003; Colombelli-Négrel et al. 2012).

The relatively low rate of ejection of the artificial nestlings might have been the consequence of lack of additional cues, besides color, required to trigger an ejection response. Shining bronze-cuckoo nestlings constantly move after they hatch, likely an adaptation to help them remove host eggs or nestlings. In contrast, the artificial nestlings were inanimate and lacked auditory cues in the form of begging calls. In addition, the timing of hatching of the nestlings has an effect on the ejection response of the host (Figure 2C) and the low ejection rate of the artificial nestlings might have been a consequence of the introduction of artificial nestlings in the nest after hatching of the host brood. The probability of ejection of artificial nestlings increased along with the frequency of ejection of cuckoo nestlings toward the end of the breeding season. This pattern suggests that nestling ejection might be a direct response to the seasonally increased presence of parasite nestlings and not the result of a parasitism risk perceived in the past, for example, due to previous experience of adult parasites approaching the nest or presence of parasite eggs in the nest, as it commonly occurs in other hosts (Briskie and Sealy 1989; Bártol et al. 2002; Avilés and Parejo 2006; Langmore et al. 2009b).

We conclude that egg and nestling ejection strategies are unlikely to co-exist as host defense behaviors in the fan-tailed gerygone. Potential visual cues such as color and luminance are not effective for the discrimination of eggs and nestlings. The evolution of an egg ejection strategy might be constrained by high costs and by physical limitations forcing acceptance of the large cuckoo egg. However, a nestling ejection strategy could have evolved because cuckoo nestlings can be more easily grasped and ejected than a large cuckoo egg. Discrimination of foreign nestlings can also be based on a combination of multiple cues such as natal down, timing of hatching, and begging calls, which further facilitates the evolution of a nestling ejection over an egg ejection strategy. Our study highlights the

importance of studying multiple cues at multiple stages of the arms race to better understand the evolution of discrimination of the brood parasite by the host.

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Author Contributions

A.A., N.J.S., K.D.T., K.U., R.G., and J.T. conceived the study. A.A., N.J.S., and K.D.T. designed the experiments. A.A., N.J.S., Y.O., and K.D.T. conducted fieldwork. A.A. analyzed the data and wrote the first draft. All authors commented on the analyses and edited the manuscript.

Ethical Standards

The Province Sud of New Caledonia issued all permits (3045-2011, 2437-2012, 2532-2013, 2801-2014, 2476-2015, 2372-2017, 3469-2018, and 2720-2019) for fieldwork. The first Warsaw Local Ethics Committee for Animal Experimentation approved the field work methods.

Conflict of Interest Statement

We declare that we have no competing interests.

Supplementary Material

“Supplementary material can be found at <https://academic.oup.com/cz/>”.

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LONG TERM STUDY

Polymorphism at the nestling stage and host-specific mimicry in an Australasian cuckoo-host arms race

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Abstract

1. Decades of research have shown that the coevolutionary arms race between avian brood parasites and their hosts can promote phenotypic diversification in hosts and brood parasites. However, relatively little is known about the role of brood parasitism in promoting phenotypic diversification of nestlings.
2. We review field data collected over four decades in Australia, New Caledonia and New Zealand to assess potential for coevolutionary interactions between the shining bronze-cuckoo (*Chalcites lucidus*) and its hosts, and how diversification at the nestling stage may be generating different subspecies.
3. The shining bronze-cuckoo is a specialist parasite of a few hosts in the family Acanthizidae. It has diversified into subspecies, of which the nestlings closely mimic the respective host nestlings in each region. Additionally, some cuckoo subspecies have polymorphic nestlings.
4. The Acanthizidae hosts have similar breeding and nesting habits and only moderately effective frontline defences against parasitism at cuckoo egg laying or at the egg stages. However, some hosts have developed highly effective defences at the nestling stage by recognising and ejecting cuckoo nestlings from the nest. As with the cuckoo nestlings, some hosts have polymorphic nestlings.
5. The coevolutionary interactions in each region suggest different evolutionary stages of the arms race in which either the parasite or the host is currently in the lead. The presence of moderately effective defences at the egg laying and egg stages might explain why some hosts do not have defences at the nestling stage.
6. The south-Pacific cuckoo – host systems are excellent models to explore the evolutionary mechanisms driving the diversification at the nestling stage in the coevolutionary arms race between avian brood parasites and their hosts.

KEYWORDS

Chalcites, coevolution, Cuculiformes:Cuculidae, geographic variation, nestling polymorphism, Passeriformes:Acanthizidae

1 | INTRODUCTION

Obligate brood parasites lay their eggs in nests of other species, their hosts, which are left to care for the foreign nestlings incurring a considerable fitness cost (Davies, 2015; Soler, 2017). This can induce an evolutionary arms race in which hosts are under selection to evolve defences against parasitism while brood parasites are under selection to evolve improved strategies to evade host defences (Dawkins & Krebs, 1979). Antagonistic behavioural interactions can often lead to phenotypic diversification (Vamosi, 2011; Yoder & Nuismer, 2010), and indeed decades of research have shown that brood parasitism can promote phenotypic diversification in several aspects of the biology of brood parasites and their hosts (reviewed in Medina et al., 2020). For example, a brood-parasitic lifestyle is associated with traits that facilitate successful parasitism, such as small body size and small eggs (Davies, 2011; Krüger & Davies, 2002), thick eggshells (Stokke et al., 2002) and plastic mating systems (Louder et al., 2019). Similarly, brood parasitism can influence the evolution of host traits such as egg (Caves et al., 2015; Øien et al., 1995; Soler & Møller, 1996) and nestling (Hauber & Kilner, 2007) phenotypes and breeding systems (Feeney et al., 2013).

Polymorphism is a common outcome of the arms race and is generally thought to result from negative frequency-dependent selection driven by host discrimination of the parasite (Cook et al., 2012; Spottiswoode & Stevens, 2012; Takahashi et al., 2010; Tanaka, 2016). For example, a common parasite morph resembling that of the host is an effective way to escape host detection, however a rare parasite morph could also escape detection if the host has not yet learned to recognise it as a parasite. Relative morph frequencies can thus oscillate over time depending on the host's discrimination ability or perception of local risk (Davies et al., 1996; Ruiz-Raya & Soler, 2020). For example, there is experimental evidence from adult common cuckoos *Cuculus canorus* that negative frequency dependent selection occurs on polymorphisms in parasite plumage (Mappes & Lindström, 2012). Females have two distinct plumage phenotypes, a grey and a rufous or hepatic form (Honza et al., 2006; Thorogood & Davies, 2012). Hosts react more aggressively towards the locally-common morph (Honza et al., 2006; Trnka & Grim, 2013), but can be induced to switch their behaviour if the alternate form becomes less rare (Thorogood & Davies, 2012). Similar plumage polymorphisms are common across Cuculidae but are more likely to occur in species that are brood parasites, suggesting that this is an adaptation to facilitate successful parasitism (Thorogood & Davies, 2013).

The factors underlying the evolution of egg polymorphism are also well understood (Honza & Cherry, 2017). A great number of host species across different avian clades recognise and reject (either by ejection or nest abandonment) the parasite egg (Medina & Langmore, 2015; Yang et al., 2020), which leads to egg mimicry by

the parasite (Brooke & Davies, 1988; Stoddard & Stevens, 2010). Host egg polymorphism can thus evolve in response to parasite mimicry because it facilitates recognition of the parasite egg (Medina & Langmore, 2016; Takasu, 2003; Yang et al., 2020). Host egg polymorphism can occur as variation between clutches (Moskát et al., 2008; Øien et al., 1995; Stokke et al., 2002), discrete polymorphism (two or more discrete egg morphs) within a host population (Liang et al., 2017; Yang et al., 2016) or as a variation in multiple egg features (eggshell colour, luminance and spot patterns) acting as individual signatures (Caves et al., 2015; Spottiswoode & Stevens, 2011). Egg polymorphism in brood parasites can occur when generalist brood parasites exploiting multiple host species specialise into host-specific lineages whose egg phenotype is a close match of the specific host they parasitise (Abernathy & Liang, 2020; Brooke & Davies, 1988; Gibbs et al., 2000; Spottiswoode et al., 2011; Starling et al., 2006; Stoddard & Stevens, 2010).

Despite decades of research, we still do not fully understand the coevolution between host and parasite nestlings. One reason for this is that much of the empirical and theoretical research has historically focused on few well-known avian parasite–host systems such as the common cuckoo from Europe and the cowbirds, genus *Molothrus*, from the Americas and their respective hosts (Kennerley et al., 2022). Many hosts in these systems have well-developed defences at the frontline (before the cuckoo egg is laid, for example mobbing the adult parasite) or egg (after the cuckoo egg is laid, for example rejection of the cuckoo egg or nest desertion) stages, but always accept the parasite nestlings (but see De Mársico et al., 2012 and Grim et al., 2003 for rejection of parasite fledglings). Therefore, nestling discrimination was assumed to be a costly defence due to the high risk of misrecognising own chicks as a parasite (Lotem, 1993). Additionally, hosts encounter parasite nestlings at lower frequencies than parasite eggs, which might relax selection for the development of host defences at the nestling stage (Britton et al., 2007; Grim, 2006). As a consequence, the nestling stage of the arms race has been less studied than the frontline and egg stages (Grim, 2011, 2017) and the selective factors and evolutionary outcomes underlying phenotypic diversification at the nestling stage of the arms race are less understood.

An example of nestling mimicry and diversification comes from the parasitic *Vidua* finches and their hosts. *Vidua* nestlings mimic gape colour and patterns, begging calls and begging postures of their estrildid hosts (Jamie et al., 2020; Payne, 2005a). However, they do not evict host nestlings or affect their survival rates (Schuetz, 2005a) and the hosts do not discriminate *Vidua* nestlings (Schuetz, 2005a, 2005b). Therefore, in these systems nestling mimicry is not the result of coevolutionary interactions between host and parasite but rather appears to be due to nestling competition to stimulate parental provisioning (Hauber & Kilner, 2007; Kilner et al., 1999). The

Australasian bronze-cuckoos (genus *Chalcites*) and their hosts, on the other hand, show evidence of a coevolutionary arms race at the nestling stage. Some hosts of bronze-cuckoos recognise and reject (Langmore et al., 2003) or eject the parasite nestling from the nest (Sato et al., 2015; Sato, Tokue, et al., 2010; Tokue & Ueda, 2010). Correspondingly, bronze-cuckoo nestlings mimic host nestlings in traits such as colour and luminance of skin, flanges and gape and presence and distribution of down feathers (Attisano et al., 2018; Langmore et al., 2011; Noh et al., 2018). Hosts discriminate the parasite nestlings via cues such as natal down, hatching order or begging calls (Attisano, Sato, et al., 2021; Langmore et al., 2003; Noh et al., 2018, 2021). Host breeding experience and risk of parasitism can also influence rejection of the parasite nestling (Langmore, Cockburn, et al., 2009). Remarkably, some of these systems also present polymorphism in both the host and parasite nestlings (Attisano, Sato, et al., 2021; Langmore et al., 2011; Sato et al., 2015).

We present a synthesis based on 40 years of studies on the biology of the shining bronze-cuckoo (*Chalcites lucidus*) and its hosts conducted in Australia, New Caledonia and New Zealand. We first describe the biology of the shining bronze-cuckoo and its hosts, highlighting commonalities and differences across the range by reviewing key aspects of these parasite–host relationships. We continue by describing host nestling mimicry by the shining bronze-cuckoo, nestling polymorphism and host defences at the nestling stage. Finally, we discuss the role of brood parasitism in promoting nestling phenotypic diversification and the evolutionary implications of nestling polymorphism in the arms race between the shining bronze-cuckoo and its hosts. The field work in New Caledonia was conducted with permits from the Province Sud Nouvelle Calédonie (3045–2011, 2437–2012, 2532–2013, 2801–2014, 2476–2015, 2372–2017, 3469–2018 and 2720–2019) and did not require ethical approval. Field work in New Zealand was conducted with permits from The Wildlife Service, Marlborough Catchment Board, The University of Canterbury Animal Ethics Committee (AEC 2010/24R) and the New Zealand Department of Conservation, *Te Papa Atawhai* (AK/15301/RES). Field work in Australia was conducted under the approval of the Australian National University Animal Experimentation Ethics Committee (Protocol Numbers F.BTZ.99.99, A2012/47 and F.BTZ.61.03, A2021/37), and Environment ACT (Licence numbers LT1999021, LT200340, LT2004112, LT2005164, LT2006229, LT2007266, LT2011509, LT2012589, LT2013667, LT2014776, LT2015842 and LT2016895).

2 | MODEL SPECIES

2.1 | The shining bronze-cuckoo

Four subspecies of the shining bronze-cuckoo are currently recognised (Payne, 2005b). The migratory *C. l. plagosus* breeds in Tasmania, south-east and south-west Australia and overwinters in the Lesser Sunda Islands and New Guinea (Friedman, 1968; Gill, 1983a). The migratory subspecies *C. l. lucidus* breeds in New Zealand and

the Chatham Islands and overwinters in the Solomon Islands and Bismarck Archipelago (Fell, 1947; Friedman, 1968). These two subspecies might come into contact during migration in Queensland outside the breeding season (Gill, 1983a; Noske, 2019), but genetic admixture is unlikely. The subspecies *C. l. layardi* forms resident populations in New Caledonia and Vanuatu. The fourth subspecies *C. l. harterti* is resident on Rennell and Bellona Islands, however little is known about its behavioural ecology, and we exclude it from our synthesis. There is no evidence of contact between migratory and resident subspecies (Friedman, 1968).

The shining bronze-cuckoo is a specialist parasite of a small group of closely related hosts in the family Acanthizidae, all of which build suspended, dome-shaped nests. The subspecies on the New Zealand mainland exclusively parasitises the grey warbler, *Gerygone igata* (Gill, 1983b, 1998), the subspecies from New Caledonia exclusively parasitises the fan-tailed gerygone, *G. flavolateralis* (Sato et al., 2015) and the subspecies from Australia is a specialist parasite of thornbills (*Acanthiza* species), particularly the yellow-rumped thornbill *A. chrysorroa* (Brooker & Brooker, 1989a). Although historical nest records in Australia reported also superb fairy-wrens *Malurus cyaneus* and splendid fairy-wrens *M. splendens* as hosts of *C. l. plagosus* (Brooker & Brooker, 1989b), multiple field studies found no parasitism of fairy-wrens (Brooker & Brooker, 1989a, 1992; Langmore & Kilner, 2007). Field experiments additionally showed that all shining bronze-cuckoo chicks transferred from thornbill nests to superb fairy-wren nests resulted in rejection of the cuckoo chicks by the fairy-wren hosts (Langmore et al., 2003). Historical records of shining bronze-cuckoo nestlings being reared by superb fairy-wrens were probably misidentifications of Horsfield's bronze-cuckoos *C. basalis*, the primary brood parasite of fairy-wrens (Payne, 2005b).

Female shining bronze-cuckoos produce dark unmarked eggs with colours ranging from olive-green to dark brown (Figure 1), which have been assumed to be cryptic in the poorly illuminated hosts' domed nests (Langmore et al., 2005; Langmore, Stevens, et al., 2009). The contrast with the host eggs is striking. All exploited hosts have eggs that are either immaculate white or whitish with brown speckles (Figure 1), but hosts usually accept foreign eggs irrespective of their coloration (Attisano, Sato, et al., 2021; Thorogood et al., 2017). Female bronze-cuckoos always remove one egg from the nest before laying their own and sometimes they can remove an egg laid by a previous cuckoo female (Gloag et al., 2014). Therefore, the dark coloration might decrease the chances of detection by other competing female cuckoos, rather than by the host (Brooker et al., 1990; Gloag et al., 2014; Thorogood et al., 2017).

The duration of the cuckoo laying season is 18–19 weeks (August – December) in Australia (Brooker & Brooker, 1989b), 13–15 weeks (October – January) in New Caledonia (Attisano et al., 2019) and 10–12 weeks (October – December) in New Zealand (Gill, 1983b). Parasitism rates vary across the three regions (Table 1) and increase over the breeding season in Australia and in New Zealand's South Island (Gill, 1983b, 1998; Medina & Langmore, 2016) are low throughout the season in New Zealand's North Island (Anderson et al., 2013) and relatively constant throughout the breeding season

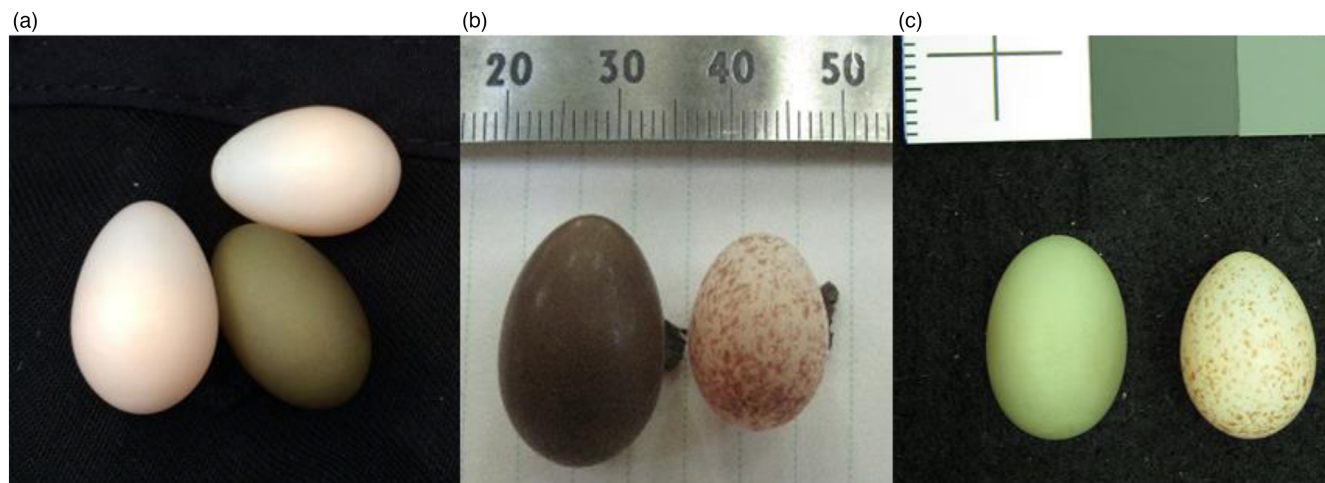


FIGURE 1 Eggs of shining bronze-cuckoos and their hosts from (a) Australia (cuckoo: olive green; yellow-rumped thornbill: unspotted white; photo by N. Langmore), (b) New Caledonia (cuckoo: dark brown; fan-tailed gerygone: whitish grey with brown speckles; photo by N. Sato) and (c) New Zealand (cuckoo: olive-green; grey warbler: whitish with brown speckles; photo by R. Thorogood).

in New Caledonia (Table 1). Multiple parasitism (i.e. when multiple cuckoo females lay single eggs in the same host nest) is common in some bronze-cuckoos (e.g. *C. minutillus*, Gloag et al., 2014). However, it is rare in the shining bronze-cuckoo: instances of two cuckoo eggs being laid in the same gerygone nest have been reported only once in New Caledonia (Attisano et al., 2019) and once in New Zealand (Briskie, 2007). The average incubation period of the cuckoo egg is shorter than that of the hosts' eggs (Attisano et al., 2019; Brooker & Brooker, 1989a; Gill, 1983b; Table 1); therefore, cuckoo nestlings usually hatch earlier than host nestlings. However, mis-timed laying can result in the cuckoo chicks hatching after the host chicks, a relatively frequent occurrence in New Zealand (Table 1). The shining bronze-cuckoo chick usually evicts all host eggs or nestlings within 1–2 days after hatching (Brooker & Brooker, 1989a; Sato et al., 2015), but eviction can occur as late as 7 days after hatching (Gill, 1983b; Table 1).

2.2 | The hosts

The nests of the Acanthizidae hosts of the shining bronze-cuckoo have a distinctive dome-shaped structure and are suspended in various trees and shrubs at heights ranging from ground level to 20m in the canopy (Attisano et al., 2019; Ebert, 2004; Gill, 1982). *G. igata* and *G. flavolateralis* are the only species building such nests in New Zealand (mainland) and New Caledonia, respectively, and no evidence of parasitism by shining bronze-cuckoos has been found in other common sympatric open-cup nesters that are potentially suitable hosts in these regions (Briskie, 2003; Gill, 1983b). In Australia, the number of potential hosts with open and domed nests is higher, however the shining bronze-cuckoo targets thornbill hosts, in particular the yellow-rumped thornbill *A. chrysorrhoa*, the nests of which closely resemble gerygone nests (Brooker & Brooker, 1989a; Medina & Langmore, 2016). Therefore, host nest location and structure might have more influence than host density or diet in determining host choice by the shining bronze-cuckoo.

The yellow-rumped thornbill from Australia is a facultative cooperative breeder and does not defend exclusive breeding territories (Ebert, 2004). On the other hand, the gerygones from New Zealand and New Caledonia form monogamous pairs and defend their breeding territory against conspecific trespassers (Attisano et al., 2019; Gill, 1982). Nest predation is the major cause of nest loss in all the hosts, with predation rates ranging from about 30% in New Zealand (Gill, 1982) to over 80% in New Caledonia and Australia (Attisano et al., 2020; Ebert, 2004). Besides nest losses due to host egg and chick eviction by the cuckoo nestling, adult female shining bronze-cuckoos also destroy 13%–16% of the active non-parasitised nests by removing host eggs or killing host nestlings (Attisano et al., 2020; Briskie, 2007). This behaviour targets nests that are too advanced in the breeding cycle and stimulates re-nesting in the hosts, thereby increasing the availability of new nests for parasitism ('farming strategy'; Soler, 2017).

The clutch size is significantly smaller in the New Caledonian host than in the hosts from New Zealand and Australia (Table 1). All the hosts lay eggs at 2-day intervals; thus, the host laying period is between 4 and 6 days in New Caledonia and 6–8 days in New Zealand and Australia. Female cuckoos increase the survival chances of their nestling by laying their egg during the host incubation period (Davies, 2015). The laying window is thus significantly shorter for the New Caledonian cuckoo, which might help explain the strong synchronicity with the host laying period (Table 1). Re-nesting after nest failure occurs in all hosts, however consecutive successful breeding attempts within the same season have been observed only in Australia and in New Zealand's South Island, whereas in the North Island and in New Caledonia host pairs have typically only one successful breeding attempt per season (Anderson et al., 2013; Attisano et al., 2019; Ebert, 2004; Gill, 1982).

All the hosts show some defences at the frontline stage. They react aggressively to adult shining bronze-cuckoos approaching the nest (Briskie, 2007) as well as to stuffed specimens of shining bronze-cuckoos presented at the nest (Attisano, Hlebowicz, et al., 2021;

TABLE 1 Details of the shining bronze-cuckoo – host systems in Australia (Brooker & Brooker, 1989a; Ebert, 2004), New Caledonia (Attisano et al., 2018, 2019; Sato et al., 2015) and New Zealand (Anderson et al., 2013; Gill, 1982, 1983b; Thorogood et al., 2017). All averages with CI.

	Australia	New Caledonia	New Zealand
Shining bronze-cuckoo subspecies	<i>C. l. plagosus</i>	<i>C. l. layardi</i>	<i>C. l. lucidus</i>
Secondary hosts	<i>A. reguloides</i>	No	No
Migratory	Yes	No	Yes
Host nestling mimicry	Yes	Yes	Yes
Nestling polymorphism (bright: dark)	91:9 (<i>n</i> = 22)	94:6 (<i>n</i> = 26)	Only dark (<i>n</i> = 20)
Average incubation (days)	13 ± 1	16 ± 1	16 ± 5
Average nestling period (days)	20 ± 1	19 ± 1	20 ± 1
Cuckoo laying relative to host laying (nests)			
Before	40%	0	0
During	53% (<i>n</i> = 15)	100% (<i>n</i> = 26)	37% (<i>n</i> = 8)
After	7%	0	63%
Cuckoo hatching relative to first host hatching (nests)			
Before	83%	80%	58%
Same day	0 (<i>n</i> = 6)	10% (<i>n</i> = 19)	0 (<i>n</i> = 12)
After	17%	10%	42%
Eviction of host eggs/nestlings relative to cuckoo hatching (nests)			
Day 1 from hatching	0	25%	0
≥ Day 2 from hatching	100% (<i>n</i> = 7)	75% (<i>n</i> = 4)	100% (<i>n</i> = 12)
Cuckoo nestlings rejected by hosts	0	86% (<i>n</i> = 28)	0
Cuckoo fledglings	92% (<i>n</i> = 24)	7% (<i>n</i> = 28)	65% (<i>n</i> = 20)
Main host	<i>A. chrysorrhoa</i>	<i>G. flavolateralis</i>	<i>G. igata</i>
Breeding system	Facultatively cooperative	Socially monogamous	Socially monogamous
Territorial	No	Yes	Yes
Rejects cuckoo eggs (ejection or nest abandonment)	Only if cuckoo laying mis-timed	No	No
Ejects cuckoo chicks	No	Yes	No
Nestling polymorphism (bright: dark)	No	75:25 (<i>n</i> = 222)	66:34 (<i>n</i> = 192)
Brood ratio (bright:dark:mixed)	All bright (<i>n</i> = 40)	69:23:8 (<i>n</i> = 132)	43:15:42 (<i>n</i> = 65)
Mean clutch size	3.2 ± 0.1 (<i>n</i> = 51)	2.0 ± 0.1 (<i>n</i> = 223)	3.5 ± 0.1 (<i>n</i> = 117)
Average incubation length (days)	17 ± 1	17 ± 1	20 ± 1
Average nestling period (days)	19 ± 1	15 ± 1	18 ± 1
Parasitism rate	30% (<i>n</i> = 106)	20% (<i>n</i> = 344)	26% (<i>n</i> = 138)
Nests with multiple parasitism	0	1	0 ^a

^aBriskie (2007) reported a case of multiple parasitism in a different field study.

Medina & Langmore, 2016). The yellow-rumped thornbill begins nesting in mid-winter, substantially earlier than congeneric and sympatric insectivorous species and before migratory cuckoos arrive in their breeding ground, which is consistent with selection for earlier breeding to escape parasitism (Medina & Langmore, 2016). A similar process might occur in the grey warbler as in the South Island breeding begins before arrival of migratory cuckoos (although not earlier than sympatric insectivores that are not parasitised); thus, earlier clutches suffer lower parasitism rates than later ones (Anderson et al., 2013).

Yellow-rumped thornbills sometimes reject cuckoo eggs laid before the host starts laying by burying these in the nest lining or they might abandon the nest if the cuckoo lays after the host has started incubation (N. Langmore, pers. obs.). They also show low rejection rates of blue artificial eggs introduced in the nest (Medina & Langmore, 2019). By contrast, gerygone hosts always accept cuckoo and artificial eggs mimicking size and colour of real host and cuckoo eggs (Attisano, Sato, et al., 2021; Gloag et al., 2014; Thorogood et al., 2017). A possible explanation for gerygone hosts being egg acceptors is that they might be unable to grasp and eject the large

cuckoo egg (Gill, 1998; Guigueno et al., 2014; Moksnes et al., 1991). In New Caledonia, the cuckoo egg is about 50% larger in volume than the host egg (Figure 1) and the fan-tailed gerygone host has a bill size comparable with small acceptor hosts of the common cuckoo in Europe and cowbirds in North America (Moksnes et al., 1991; Rasmussen et al., 2010). Indeed, fan-tailed gerygones always accept artificial eggs matching the size of real eggs but frequently eject artificial eggs smaller than real eggs even if these mimic the colour of host or parasite eggs, thus suggesting that the small bill size might prevent ejection of the large cuckoo eggs (Attisano, Sato, et al., 2021). Another explanation is that by accepting the cuckoo egg the host reduces the chances of losing an additional host egg during multiple parasitism attempts by different cuckoo females (Sato, Mikami, & Ueda, 2010).

Frontline and egg defences in these hosts are thus only partly or not at all effective in preventing parasitism. This might have been instrumental in promoting an escalation to the nestling stage as the next step in these arms races (Grim, 2017; Langmore et al., 2003).

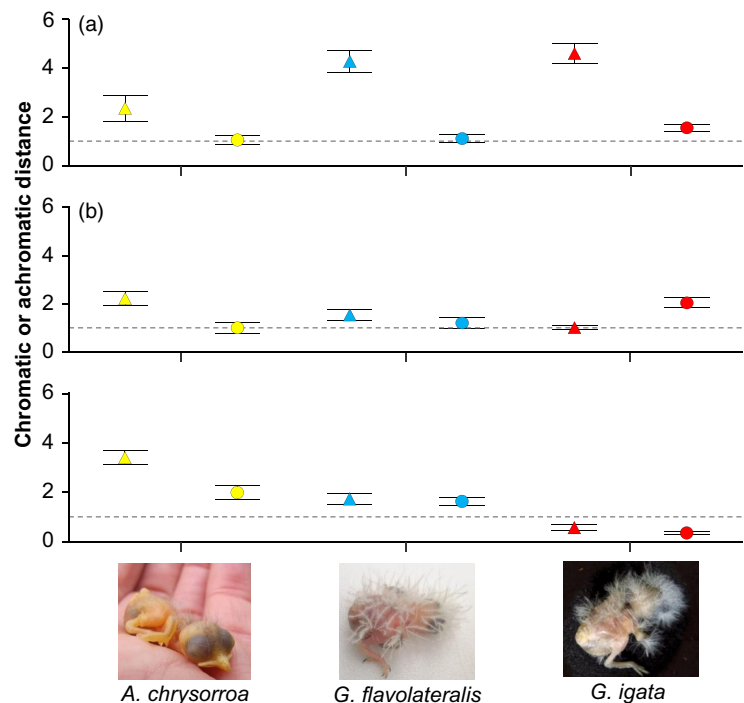
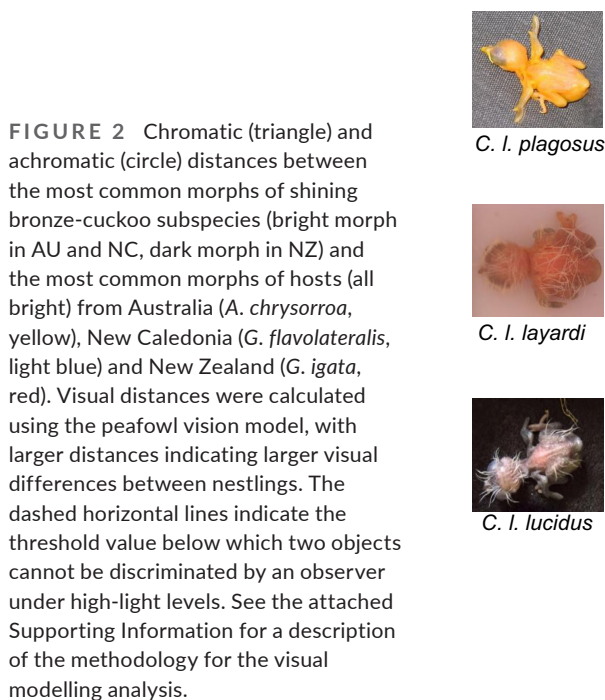
3 | NESTLING MIMICRY, POLYMORPHISM AND DISCRIMINATION

3.1 | Nestling mimicry and polymorphism in parasite nestlings

Discrimination of the parasite egg by the hosts favours selection of host egg mimicry in brood parasites (Honza & Cherry, 2017). Similarly, discrimination of the parasite nestling by hosts of bronze-cuckoos (Langmore et al., 2003; Sato, Tokue, et al., 2010; Tokue & Ueda, 2010) has favoured selection of host nestling mimicry in

several bronze-cuckoo species (Langmore et al., 2011). Some hosts of bronze-cuckoos also discriminate the begging call of the parasite nestling (Colombelli-Négrel et al., 2012; Langmore et al., 2003; McLean & Waas, 1987), which in turn has favoured selection of begging call mimicry by the parasite (Anderson et al., 2009; Langmore et al., 2008; Noh et al., 2021; Ranjard et al., 2010).

The shining bronze-cuckoo also has geographic variation in visual mimicry with nestlings of each subspecies closely resembling the respective local host in traits such as colour and luminance of the skin and mouth flanges (Figure 2 and Supplementary Material). McGill and Goddard (1979) found a similar difference in cuckoo nestling coloration among subspecies of the little bronze-cuckoo *C. minutillus*. Besides coloration, the shining bronze-cuckoo nestlings of each subspecies also usually mimic their respective hosts in regard to the presence of natal down; cuckoo nestlings from New Caledonia and New Zealand have sparse down feathers which mimic the natal down of the host nestlings (Figure 3). However, cuckoo nestlings in Australia are usually naked or have a small number of short, fine filaments on the head, whereas host nestlings have a longer, fine down on the head and back (Langmore et al., 2011). Usually, Cuculinae nestlings are naked at hatching (Payne, 2005b), however natal down is a common trait of bronze-cuckoos parasitising gerygone hosts (Langmore et al., 2011; Tokue & Ueda, 2010). Remarkably, shining bronze-cuckoo nestlings can also occur in two distinct skin colour morphs within a subspecies, typically a common 'bright' and a rare 'dark' (melanic) morph (Figure 3). The occurrence of polymorphism in shining bronze-cuckoos varies across its range, as it has been observed only in Australia and New Caledonia (Langmore et al., 2011; Sato et al., 2015), but not in New Zealand where Gill (1983b) only observed a dark morph (Table 1). There is therefore evidence of the formation of host-specific lineages in the shining bronze-cuckoo,



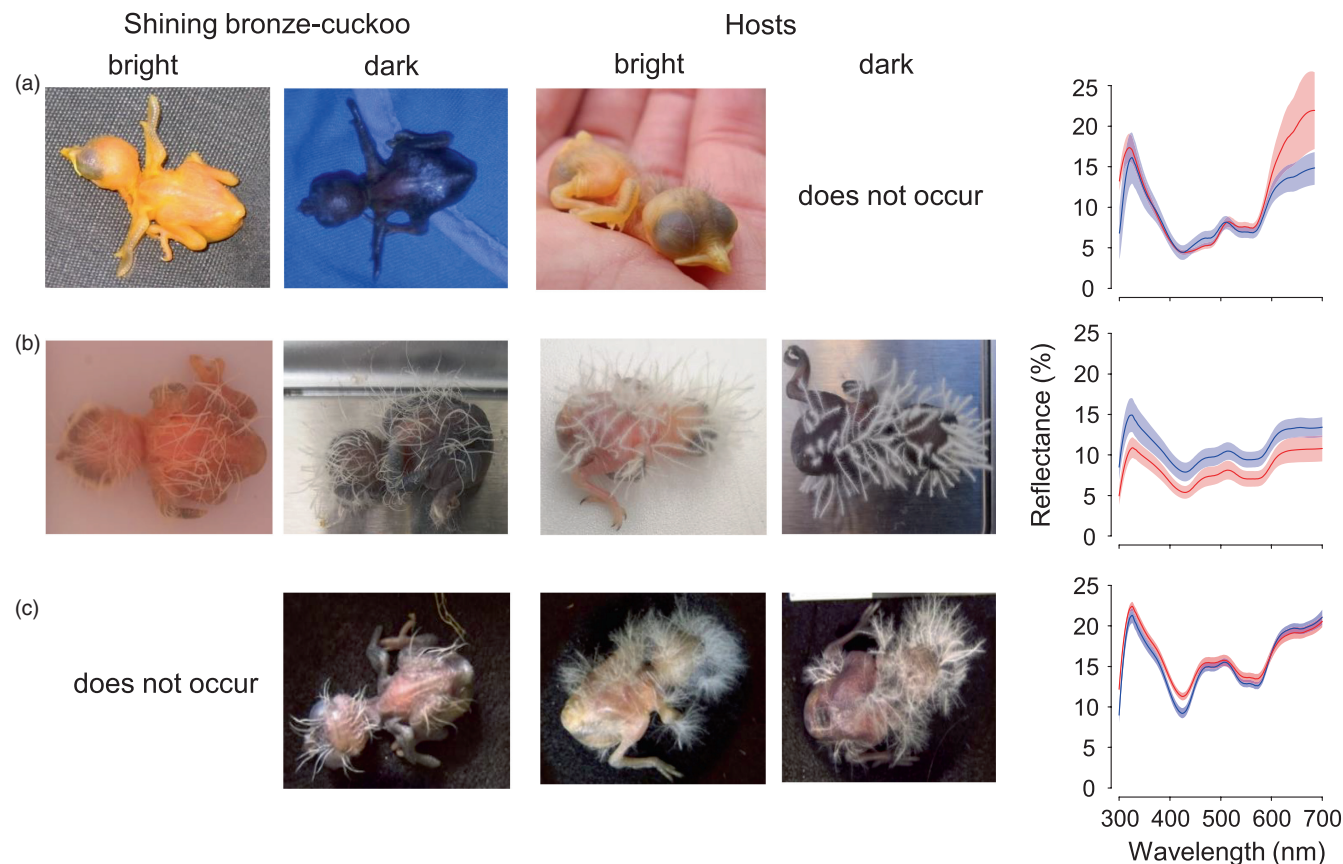


FIGURE 3 Representative photos and reflectance spectra showing the phenotypic variation in nestlings of shining-bronze cuckoos and their hosts in (a) Australia (modified from Langmore et al., 2011; photos by N. E. Langmore), (b) New Caledonia (modified from Sato et al., 2015; photos by A. Attisano) and (c) New Zealand (data from M. G. Anderson and R. Thorogood, photos by R. Thorogood). All nestlings were newly hatched (day 0–1), except (c) in which host nestlings were 4 days old. Lines in graphs are mean reflectance spectra (shaded areas are 95% CI) of the most common morph of nestlings of the shining bronze-cuckoo (red lines, bright morph in AU and NC, dark morph in NZ) and the respective host (blue lines, all bright morphs) from each region.

as the nestlings of each cuckoo subspecies appear more similar to their main hosts than they are to each other (Figures 2 and 3 and Supplementary Material).

3.2 | Polymorphism in host nestlings

One of the most intriguing aspects of the shining bronze-cuckoo – host systems is that hosts also have polymorphic nestlings with a common ‘bright’ and a rare ‘dark’ (melanic) morph (Figure 3). As with the cuckoo, polymorphism varies regionally: gerygone hosts from New Caledonia and New Zealand have polymorphic nestlings, whereas yellow-rumped thornbill nestlings from Australia are monomorphic (Figure 3). In addition, while the New Caledonian host is distinctly dimorphic, colour variation in the New Zealand host is less evident yet clearly noticeable to a human observer. In New Zealand, the two host nestling morphs progressively darken with age (Gill, 1983c), whereas in New Caledonia only the bright morph becomes progressively darker until the two morphs are indistinguishable from each other around the age of 9–10 days (Attisano et al., 2018). The two morphs of the New Caledonian host also have

similar growth and survival rates, with no evident effect of skin coloration on nestling condition (Attisano et al., 2019).

The two nestling morphs can co-exist in the same brood (Gill, 1983c; Sato et al., 2015). The overall ratio of bright to dark host nestlings is similar in New Caledonia and New Zealand, but mixed broods are relatively more common in the New Zealand host and the brood coloration varies across sites within both regions (Table 1 and Supplementary material). At least for the New Caledonian host, extra-pair copulation does not influence the proportion of mixed broods (Bojarska et al., 2018) and population genetic analyses confirm that the observed host chick polymorphism does not diverge from a Hardy–Weinberg equilibrium, particularly when assuming a dominant dark-morph allele (Sato et al., 2015).

3.3 | Host defences at the nestling stage

In Australia, all cuckoo nestlings that hatched in nests of yellow-rumped thornbills evicted host eggs or nestlings and were reared by the hosts. There is thus no indication of discrimination of the parasite nestling (Table 1).

In New Zealand, a total of 20 cuckoo nestlings hatched in nests of grey warblers and evicted host eggs or nestlings, and 13 cuckoos were reared by the host (Table 1). Of the seven cuckoo nestlings that failed to fledge, four died from nest depredation but the cause of death of the other three cuckoo nestlings was uncertain. In one case, a cuckoo nestling was dead in the nest after a sudden change to cold and wet weather and with no signs of injury, however we cannot rule out that the death resulted from nest abandonment by host parents. Finally, two other nests, each previously containing a cuckoo nestling (3–7 days old), were found intact but empty at last inspection. It is possible that these nestlings were depredated by adult shining bronze-cuckoos (Briskie, 2007), but we also cannot exclude the possibility that host parents removed the parasite from the nest. In summary, field data show that the grey warbler host does not usually discriminate and eject the cuckoo nestling, but any rejection of the cuckoo nestling cannot yet be ruled out without an experiment manipulating nestling mimicry.

By contrast, in New Caledonia most cuckoo nestlings that hatched in fan-tailed gerygone nests were ejected by the host within 24–48 h from hatching before they could evict any host eggs or nestlings (Table 1). The discrimination ability of the host is very accurate: they ejected the parasite even when it was the only chick in the nest and never ejected host chicks in non-parasitised or parasitised nests (Attisano et al., 2018; Sato et al., 2015). Both parents can eject the parasite nestling: in three cases, this was the host mother, in another three cases the host father and in one case both parents seemingly collaborated in ejecting the parasite nestling. The parasite nestling was ejected whether or not it matched the host brood colour (Attisano et al., 2018; Sato et al., 2015). Field experiments show that nestling down, hatching order and begging calls might be more important cues for the recognition of the parasite than chick colour (Attisano, Sato, et al., 2021). Host acceptance of the shining bronze-cuckoo nestlings in New Caledonia is relatively low, as only 14% of the cuckoo hatchlings ($n = 28$) were accepted by the host parents, of which only two fledged (Table 1).

4 | DISCUSSION

4.1 | Host-specific lineages in the shining bronze-cuckoo

Chalcites bronze-cuckoos are already known for the exquisite mimicry exhibited by their nestlings (Langmore et al., 2011), but our synthesis adds a further level of complexity in these intriguing arms races by highlighting the remarkable phenotypic diversification of the shining bronze-cuckoo's nestlings across its range. The shining bronze-cuckoo is a specialist brood parasite of a small number of passerines, some of which discriminate and reject the parasite nestling. The choice of hosts might be constrained by their availability, for example only one host exists in New Caledonia and mainland New Zealand, by competition for hosts with other bronze-cuckoo species, for example between *C. minutillus* and *C. basalis* in Australia,

or by specific habitat or diet requirements (Payne, 2005b). It is well known that discrimination of the parasite egg by the host can lead to parasite egg mimicry and diversification into host-egg-specific lineages (Abernathy & Liang, 2020; Gibbs et al., 2000; Spottiswoode et al., 2011; Starling et al., 2006). Therefore, the shining bronze-cuckoo – host systems fulfil the expectations of selection for the evolution of specialised host-specific lineages at the nestling stage of the arms race much in the same way that host discrimination drives parasite egg mimicry and diversification.

No study yet has looked at the genetic relationships among the shining bronze-cuckoo subspecies, whereas the population genetic structure and host-specific lineages of two closely related species, the Horsfield's bronze-cuckoo (*C. basalis*) and the little-bronze-cuckoo (*C. minutillus*), have received more attention. The Horsfield's bronze-cuckoo shows no evidence of host-specific diversification (Joseph et al., 2002). It has instead evolved a 'Jack-of-all-trades' strategy that appears to have relaxed selection for host-specific lineages: it has evolved an egg that is somewhat similar to the eggs of several hosts (Feeney et al., 2014), a two-tone nestling colour that is somewhat similar to nestlings of various hosts (Langmore et al., 2011) and a begging call that can be modified to match that of the relevant host (Langmore et al., 2008). Australian populations of the little bronze-cuckoo are not completely genetically separated (Joseph et al., 2011), but present nestling diversification that suggests the presence of host-specific lineages (McGill & Goddard, 1979). The shining bronze-cuckoo has a wider geographic range and subspecies that are spatially separated in distinct geographic regions, which could result in genetic isolation and a more marked differentiation than in other closely related species.

4.2 | Coevolutionary arms race and nestling polymorphism

Mimicry and host-specific lineages at the egg and nestling stages might be shaped by similar selective factors and have similar roles in the coevolutionary interactions between brood parasites and their hosts (Honza & Cherry, 2017; Tanaka, 2016). However, the role of nestling polymorphism in the arms race is not as clear as that of egg polymorphism. Nestling polymorphism is rare in birds (Kilner, 2006), whereas egg polymorphism is relatively common (Yang et al., 2020). A few studies have reported discrete polymorphism in melanin or carotenoid-based plumage coloration in older nestlings or juveniles (Galván et al., 2010; Kapun et al., 2011; Rohwer et al., 2012; Roulin et al., 2016), however, the case of shining bronze-cuckoos and their gerygone hosts is, to our knowledge, the only known example of discrete skin, but not plumage, polymorphism in hatchlings, and of nestling polymorphism occurring in both the brood parasite and the host.

Phenotypic diversification and polymorphism are known in brood parasites as a consequence of their parasitic habits (Medina & Langmore, 2015). The rare dark morph of the Australian cuckoo subspecies might indicate a specialisation to a host with unknown nestling colour (e. g. Tasmanian thornbill, *Acanthiza ewingii*). The

New Caledonian cuckoo subspecies started parasitising its single host within the last 1.7 my (Nyári & Joseph, 2012) and the nestling polymorphism might have originated before the separation from the Australian subspecies or have parallelly been developed in more recent times. The absence of polymorphism in the New Zealand cuckoo might be due to the loss of a second morph or because this was not observed during field studies due to small samples and its rarity. However, in all regions we found that nestlings of each cuckoo subspecies have a remarkable resemblance to the main host (Figures 2 and 3 and Supplementary Material). Furthermore, the common bright cuckoo morph in New Caledonia mimics several visual features of both host morphs (Attisano et al., 2018), which might indicate selection for an intermediate parasite morph similar to the intermediate egg and nestling phenotypes of the generalist common cuckoo (Stoddard & Stevens, 2010) and Horsfield's bronze-cuckoo (Feeney et al., 2014; Langmore et al., 2011). Such a strategy should return higher pay-offs due to the overall high frequency of bright host nestlings, of which the bright cuckoo is a closer match, and still offer some competitive advantage when the parasite does not match the host brood colour. Therefore, the rarity of the dark morphs in Australia and New Caledonia and the monomorphism in New Zealand might indicate selection towards improved mimicry of the host nestlings rather than polymorphism.

Host nestling polymorphism fits with theoretical predictions that offspring phenotypes dissimilar from the mimetic parasite should improve the capability for discrimination by the host (Takasu, 2003; Tanaka, 2016); however, the nestling phenotype might be a less reliable discrimination cue than the egg phenotype. First, the egg phenotype is maternally inherited (Gosler et al., 2000), whereas the nestling phenotype is a result of the genetic contribution of both parents (Roulin & Dijkstra, 2003). For example, in New Caledonia each adult host can experience a brood colour change across seasons due to partner replacement or extra-pair copulations (Bojarska et al., 2018), which also means that the frequency of mixed broods can be low but cannot reach zero in this host. Second, egg discrimination in rejector hosts is improved by increased within-nest uniformity and between-nest variability of egg phenotypes (Moskát et al., 2008; Stokke et al., 2002), however mixed broods of the two gerygone hosts challenge this assumption and should in theory lead to more recognition errors and rejection of own offspring by mistake. The frequency of mixed broods varies among and within regions which might suggest a disadvantage of mixed broods in areas with high parasitism pressure and thus selection for monomorphic broods of either morph. Third, the female is usually the sex responsible for the discrimination and rejection of the parasite egg as she can imprint on her own egg phenotype or compare the foreign egg with her own (Davies, 2015; Grim, 2017). However, in the fan-tailed gerygone both sexes can discriminate and eject the parasite nestling. Thus, imprinting on their own nestling phenotype is advantageous only if the pair produces the same nestling phenotype, which is not always the case in this host. Therefore, nestling coloration should not be the main cue for the discrimination of the parasite. Experimental evidence indeed shows that fan-tailed gerygones discriminate foreign

nestlings mainly based on cues such as down feathers, hatching order and begging calls (Attisano, Sato, et al., 2021).

Nestling polymorphism might also be related to environmental factors, for example skin coloration might be influenced by UV radiation levels that are potentially harmful for the nestlings. Testing this hypothesis will require more work as little is known about the possible protective role of skin melanisation against UV radiation in nestlings (Galván & Solano, 2016). Melanisation is also linked to immune response and fitness in birds (Chakarov et al., 2008; Gangoso et al., 2015), however the condition of nestlings does seem to be unrelated to skin melanisation in the fan-tailed gerygone (Attisano et al., 2019). Further work is thus needed to understand if and how environmental factors and physiology contribute to nestling polymorphism in these host species.

In New Caledonia, nestling polymorphism might have decreased the rate of recognition errors of the parasite during early stages of the arms race. However, it gradually lost its importance as the main discrimination cue due to the cuckoo achieving improved mimicry and polymorphism, leading to a finer discrimination based on a combination of multiple cues (Attisano et al., 2018; Attisano, Sato, et al., 2021). Polymorphism can then persist in the host population because it bears no additional costs to nestling growth and survival. In New Zealand, the arms race might be at an early stage as the host morphs are not yet sufficiently dissimilar from the parasite nestling to allow host parents to reliably discriminate the parasite (Figure 3 and Supplementary Material). Further support for different evolutionary stages of the arms race also come from the fact that the cuckoo accurately mimics the begging calls of the grey warbler in New Zealand (Anderson et al., 2009; McLean & Waas, 1987; Ranjard et al., 2010), whereas the mimicry is imperfect in New Caledonia (Attisano, Sato, et al., 2021).

4.3 | Host defences at the nestling stage

The nestling mimicry by the Australian cuckoo subspecies suggests the possibility that a thornbill host was able to discriminate the parasite nestling at some point in the past but nowadays it can no longer accurately discriminate the parasite from its own chicks. Instead, it developed moderately effective anti-parasitism defences at the nest-building and egg stages (Medina & Langmore, 2016), which might have further relaxed selection for improved nestling recognition (Britton et al., 2007; Grim, 2006).

Besides the fan-tailed gerygone from New Caledonia, two other gerygone species from Australia, the large-billed *G. mag-nirostris* and the mangrove gerygone, *G. laevigaster*, which are parasitised by the little bronze-cuckoo, recognise and eject parasite nestlings (Sato, Tokue, et al., 2010; Tokue & Ueda, 2010). These three gerygone nestling ejector species belong to the same evolutionary branch (Nyári & Joseph, 2012), thus suggesting a common origin for this host defence behaviour. True recognition of the parasite occurs in the large-billed gerygone and fan-tailed gerygone hosts (Attisano, Sato, et al., 2021; Noh et al., 2018).

However large-billed gerygones sometimes eject their own nestlings by mistake (Noh et al., 2018; Sato, Tokue, et al., 2010; Tokue & Ueda, 2010), whereas fan-tailed gerygones do not (Attisano et al., 2018; Sato et al., 2015). The misrecognition errors of the large-billed gerygone host might depend on the fact that the little bronze-cuckoo is the gerygone specialist in Australia and nestling mimicry can be so accurate as to sometimes fool the host. On the other hand, the shining bronze-cuckoo is not a gerygone specialist in its Australian range meaning the fan-tailed gerygone might only be a suboptimal host, yet the only one building a dome-shaped nest in New Caledonia, and the less accurate mimicry allows the host to always recognise its own nestlings. The New Caledonian cuckoo subspecies is also a year-round resident; thus the parasitism pressure is evenly spread across the entire breeding season and the host cannot escape parasitism via changes in the breeding phenology. Coupled with the lack of effective defences at the egg laying stage, these factors might have strengthened selection for accurate nestling discrimination in the fan-tailed gerygone host.

The grey warbler from New Zealand is closely related to the three gerygone ejector species and separated from them about 100,000 years ago (Nyári & Joseph, 2012). Therefore, the arms race in New Zealand might be at a relatively early stage in which enough selective pressure promoted the parasite's visual and auditory mimicry but not nestling dimorphism and recognition of the parasite nestling by the host. It is also possible that grey warblers have some defences at the nestling stage that do not involve ejection of the parasite, for example they might abandon nests containing a single parasite nestling as other bronze-cuckoo hosts do (Langmore, Cockburn, et al., 2009), but our field evidence does not conclusively support this. Grey warblers also escape parasitism thanks to their breeding phenology as populations on the North Island and earlier breeders in the South Island of New Zealand suffer low parasitism rates (Anderson et al., 2013; Gill, 1983b). This might suggest relaxed selection for nestling discrimination in this host.

5 | CONCLUSIONS

The shining bronze-cuckoo – host systems are promising models for coevolutionary research as they can contribute to significant advancements of current theories of the nestling stage of the arms race. We suggest some possible research avenues that could contribute greatly to our understanding of these systems. First, there is the need to clarify the phylogenetic relationships within the shining bronze-cuckoo clade and understand if the variation in nestling appearance is due to the presence of host-specific lineages or to genetically distinct cuckoo species. Second, detailed field studies of other members of the *Gerygone* clade are needed to assess the variation in host defences and the occurrence of nestling polymorphism across members of the group. The ancestral nature of the parasite ejection behaviour can only be supported by confirming its occurrence in a larger number of *Gerygone* species. Additionally,

gerygones show specialisation to different types of habitats over their wide geographic range (Keast & Recher, 1997), thus factors dependent on local ecological conditions (for example temperature, solar radiation or nest microclimate environment) could potentially influence the nestling phenotype. Therefore, gerygones are excellent models for comparative analyses of the wider ecological factors shaping the evolution of nestling polymorphism and defences at the nestling stage of the arms race. Third, the study of the genetic and physiological mechanisms underlying nestling skin polymorphism could help in understanding the selective factors contributing to the expression of the trait. Molecular (genomics, proteomics) and physiological (stress response assays) techniques have so far been employed in the study of nestling plumage polymorphism (Gangoso et al., 2015; Roulin & Dijkstra, 2003), yet no study has been published on species with skin polymorphic nestlings. Finally, a more careful investigation on the presence of nestling polymorphism in hosts, including those not belonging to the genus *Gerygone*, and non-hosts is also needed. Nestling polymorphism might have been overlooked due to an historical imbalance of studies focusing on the egg stage rather than the nestling stage of the arms race. Examples of host defences at the nestling stage have been found in multiple hosts (reviewed in Grim, 2017), thus researchers should be aware that nestling polymorphism could be a possible outcome of the arms race. At the same time, evidence of nestling polymorphism in non-hosts would allow to confirm or deny its role within an arms race and better understand its evolutionary causes.

AUTHOR CONTRIBUTIONS

Alfredo Attisano, Brian J. Gill, Michael G. Anderson, Roman Gula, Naomi E. Langmore, Nozomu J. Sato, Keita D. Tanaka, Rose Thorogood, Keisuke Ueda and Jörn Theuerkauf designed the studies; Alfredo Attisano, Brian J. Gill, Michael G. Anderson, Naomi E. Langmore, Yuji Okahisa, Nozomu J. Sato, Rose Thorogood collected the data; Alfredo Attisano led the writing of the manuscript; Alfredo Attisano, Brian J. Gill, Michael G. Anderson, Roman Gula, Naomi E. Langmore, Keita D. Tanaka, Rose Thorogood and Jörn Theuerkauf contributed to the writing; all authors gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data used to generate Figures 2 and 3 and additional figures and tables in the Supporting Information is available from Dryad Digital Repository <https://doi.org/10.5061/dryad.95x69p8pf> (Attisano et al., 2022).

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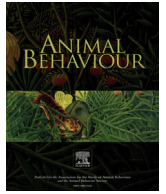
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Begging call mimicry and formation of host-specific lineages in the shining bronze-cuckoo, *Chalcites lucidus*

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Antagonistic coevolutionary interactions between avian brood parasites and their hosts can lead to selection for the discrimination of parasites by their host via visual or acoustic signals. Therefore, nestlings of some brood parasites may be under selection to evolve visual and vocal mimicry of host nestlings to avoid discrimination by host parents. Some brood parasites diversify into host-specific populations by mimicking visual features of the host offspring (either eggs or nestlings). However, whether a similar mechanism applies to the begging calls of their hosts remains unclear. In this study, we analysed the begging calls of three subspecies of the shining bronze-cuckoo, which present visual mimicry of three different host species (family Acanthizidae) that vary in their ability to discriminate parasite nestlings. We found that the begging calls of each cuckoo subspecies match the respective host more closely than any of the other cuckoo subspecies. Therefore, selection for coevolutionary diversification appears to overcome phylogenetic constraints on cuckoo begging call structure. The coevolutionary interactions in these parasite–host systems have promoted the selection for refined host mimicry by the cuckoo and the formation of geographically isolated evolutionary units across the range of the species.

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Obligate avian brood parasites lay their eggs in nests of other species, their hosts, which are left to care for foreign nestlings, thereby incurring high fitness costs (Soler, 2017a). Hosts and parasites can be involved in a coevolutionary arms race in which host discrimination of the parasite can lead to adaptations by the parasite to exploit the host, followed by adaptations by the host to avoid parasitism (Davies, 2011; Dawkins & Krebs, 1979). Agonistic interactions within the arms race can occur at all stages of the host breeding cycle, starting from before the parasites laid eggs to the fledging stage (Soler, 2017a). These agonistic coevolutionary interactions can lead to phenotypic diversification (Langmore et al., 2024), and several studies have shown that the arms race has promoted phenotypic diversification in several aspects of the biology of hosts and parasites (Medina et al., 2020). Still little is known about diversification occurring at the nestling stage, as this has received relatively little attention compared to other stages of

the host breeding cycle (Grim, 2017). However, in a few host–parasite systems, the arms race has reached remarkable levels of sophistication in the ability of hosts to discriminate parasite nestlings and in the ability of parasites to accurately mimic young hosts (Attisano et al., 2023; De Mársico et al., 2019; Jamie et al., 2020; Langmore et al., 2003; Sato et al., 2015).

Begging by nestlings plays a fundamental role in offspring–parent communication by signalling nestling needs, thereby influencing parental provisioning (Kilner, 2002). Nestlings of brood parasites can break into this communication system by either exploiting host preferences for certain nestling signals such as loud begging or by mimicking the structure of the host begging calls (Soler, 2017b). The selective factors determining the strategy of parasites depend on the lifestyle of parasites and the ability of hosts to discriminate the parasite nestling (Jamie & Kilner, 2017). Nestlings of generalist brood parasites exploiting non-discriminating hosts produce exaggerated begging calls that tune into the foster parents' sensorial biases of providing food to chicks with a larger size or louder calls, for example, common cuckoo, *Cuculus canorus* (Kilner et al., 1999; Samaš et al., 2020), great spotted cuckoo, *Clamator glandarius* (Roldán et al.,

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2013), or shiny cowbird, *Molothrus bonariensis* (Glog & Kacelnik, 2013; Tuero et al., 2016). Nestlings of specialist brood parasites exploiting discriminating hosts produce begging calls mimicking host calls, thereby tricking the foster parents into accepting and feeding the parasite chick as one of their own, for example, bronze-cuckoos, *Chalcites* spp. (Anderson et al., 2009; Attisano et al., 2021; Langmore et al., 2008; Noh et al., 2021), and screaming cowbird, *Molothrus rufoaxillaris* (Lama et al., 2022; Ursino et al., 2018). By contrast, in brood parasitic *Vidua* finches, host parents do not discriminate the parasite (Schuetz, 2005), yet parasite nestlings mimic the begging behaviour of the host (Jamie et al., 2020). In these systems, mimicry might have resulted from nestling competition for parental provisioning rather than a coevolutionary arms race between the host and the parasite (Hauber & Kilner, 2007).

Whether begging calls of nestlings of different host races of a parasite match those of the specific hosts and if this variation represents host specialization remain unknown (Wang et al., 2020). The common cuckoo has host races with distinct eggs matching those of the specific hosts they parasitize (Gibbs et al., 2000; Stoddard & Stevens, 2010). However, nestlings of these host races do not match the begging calls of their hosts, thereby showing no host-race specialization of begging calls (Samaš et al., 2020). On the contrary, common cuckoo nestlings maximize parental provisioning by producing calls that are always exaggerated regardless of the host size or species (Kilner et al., 1999; Soler, 2017b). Similarly, the begging calls of the nestlings of great spotted cuckoo, a specialist parasite of Corvidae, do not match the begging calls of different hosts by which they are raised (magpie, *Pica pica*, carrion crow, *Corvus corone*, or jackdaw, *Corvus monedula*). However, they adjusted their call rate to maximize the provisioning rate from foster parents (Roldán et al., 2013).

Bronze-cuckoos and their hosts (family Maluridae and Acanthizidae) from Australasia and SE Asia are the best examples of an arms race at the nestling stage. In these systems, bronze-cuckoo nestlings evict the host eggs or young and monopolize the nest; however, some hosts discriminate and reject the parasite nestlings, thereby promoting selection for visual (Attisano et al., 2018; Langmore et al., 2011; Noh et al., 2018) and vocal mimicry (Anderson et al., 2009; Attisano et al., 2021; Langmore et al., 2003, 2008; Noh et al., 2021) of the host nestlings by the parasite nestlings. In this group, we found examples of begging call mimicry by generalist and specialist brood parasites. The Horsfield's bronze-cuckoo *Chalcites basalus* is a generalist parasite that uses Maluridae as primary hosts and Acanthizidae as secondary ones (Payne, 2005). The cuckoo nestlings innately produce begging calls mimicking those of the primary host, the superb fairywren, *Malurus cyaneus*. However, if they find themselves in nests of a secondary host, the buff-rumped thornbill, *Acanthiza reguloides*, they can modify their calls to match those of the new host (Langmore et al., 2008). This process involves social shaping by the host parents with the cuckoo modifying call features that increase the likelihood of obtaining food from the host (Langmore et al., 2008). This plasticity in begging calls might reduce selection for host-race formation, which is supported by the lack of a phylogenetic structure of the Australian population of Horsfield's bronze-cuckoo (Joseph et al., 2002). By contrast, the little bronze-cuckoo, *Chalcites minutillus* is a specialist parasite that exploits several species of *Gerygone* hosts across its range (Noh et al., 2021; Payne, 2005). Recent evidence demonstrates that this species selection for mimicry of host nestlings has driven genetic and phenotypic divergence corresponding to host preference (Langmore et al., 2024). Consequently, nestling phenotypes of little bronze-cuckoo subspecies track divergent host nestling morphologies across their geographical range (Langmore et al., 2024). The nestlings of little bronze-cuckoo appear to be specialists, not only with regard to

visual mimicry, but also in terms of vocal mimicry; they produce begging calls that match those of their main host, the large-billed gerygone, *Gerygone magnirostris*. However, unlike the Horsfield's bronze-cuckoo, their begging calls are not altered when raised by a secondary host, which suggests a fixed development of the calls (Noh et al., 2021).

The shining bronze-cuckoo has the widest distribution across the Australasian region from New Zealand to New Guinea (Payne, 2005). To date, no study has looked at the genetic relationships among subspecies; however, some cuckoo subspecies match the visual features of the nestlings of the main hosts across their geographical range: the fan-tailed gerygone, *Gerygone flavolateralis*, in New Caledonia, the grey warbler, *Gerygone igata*, in New Zealand and the yellow-rumped thornbill, *A. chrysorrhoa* in Australia (Attisano et al., 2023; Langmore et al., 2024). Compared with the hosts from Australia and New Zealand (Attisano et al., 2023), the host from New Caledonia rejects cuckoo nestlings (Attisano et al., 2023; Sato et al., 2015). The begging calls of shining bronze-cuckoo nestlings also match those of the two gerygone hosts in New Caledonia and New Zealand (Anderson et al., 2009; Attisano et al., 2021). However, it is unclear whether the call matching occurs only in relation to these two closely related gerygone hosts or is rather a general pattern resulting from selection for host specialisation of each cuckoo subspecies, similar to the selection for visual mimicry found across the range.

In this study, we investigated if nestlings of the three subspecies of shining bronze-cuckoo from Australia (*C. l. plagosus*), New Caledonia (*C. l. layardi*) and New Zealand (*C. l. lucidus*) present mimicry of begging calls of their respective hosts. We expect that the begging calls of each cuckoo subspecies of each cuckoo subspecies should match the local host more closely than the other hosts or cuckoo subspecies from other regions. Overall, the begging call structure should reflect coevolutionary interactions rather than the phylogeny of the cuckoo subspecies. Moreover, accurate matching of the host begging calls by cuckoo should increase the chances of recognition errors, thereby decreasing or eliminating rejection of the cuckoo nestlings by the host. Therefore, cuckoo nestlings likely match the begging calls of the non-rejecter hosts in Australia and New Zealand more closely than the rejecter host from New Caledonia. Finally, closely related hosts likely have higher similarity in their begging calls. Consequently, cuckoo subspecies parasitizing these hosts should have begging calls that are more similar to one another. Therefore, the begging calls of the two shining bronze-cuckoo subspecies exploiting gerygone hosts should be more similar to each other than to the begging calls of the subspecies exploiting the thornbill host.

METHODS

The begging calls of three shining bronze-cuckoo subspecies and their respective hosts from Australia, New Caledonia and New Zealand were recorded from July to January, which corresponds to the breeding season of hosts and cuckoos in the Australasian region (Attisano et al., 2023). The active nests at our field sites were regularly monitored at 2-day intervals, thereby recording the laying, hatching and fledging dates with a precision of 2 days. The timing was assessed as the average between two nest checks, thereby resulting in an uncertainty of ± 1 day. Begging calls were recorded from 2005 to 2009 in an open eucalypt woodland in Australia (Campbell Park, 35°16'S, 149°10'E), from 2005 to 2007 in forest and scrub areas in New Zealand (Tawharanui Regional Park, North Island: 36°22'S, 174°50'E) and from 2015 to 2023 at three sites on the main island (Grande Terre) of New Caledonia in rain-forest (Parc Provincial des Grandes Fougères, 21°39'S, 165°46'E), open forest, thickets (Farino and surrounding, 21°40'S, 165°46'E),

open dry forest and savannah (Domaine de Deva, 21°36'S, 165°24'E). Field methods to monitor host nests and cuckoo breeding are described elsewhere (Anderson et al., 2013; Attisano et al., 2019; Medina & Langmore, 2019).

A total of 23 recordings were collected from 14 cuckoo nestlings aged between 3 and 14 days: 10 recordings from 8 *C. l. plagosus* from Australia (AU_parasite) and 13 recordings from 6 *C. l. layardii* from New Caledonia (NC_parasite). We could not record young cuckoo nestlings *C. l. lucidus* in New Zealand (NZ_parasite), but we included four recordings from one 19-day-old cuckoo nestling and three 20-day-old cuckoo fledglings (recorded on the fledging day). Shining bronze cuckoos young fledge around 20 days after hatching and remain close to the nest for at least 1–2 days while being fed by host parents (Gill, 1983; Attisano et al., 2023). Preliminary visual analysis of the spectrograms of the begging call recorded from these young cuckoos revealed close similarity in call structure among these cuckoos and with host nestlings aged 10–12 days (Supplementary material, Fig. S3). Two other studies found a strong similarity in call structure of young cuckoo fledging with younger host nestlings in New Zealand (Anderson et al., 2009; Ranjard et al., 2010). Therefore, if call mimicry of the host by NZ_parasite occurred, we expected to be able to detect it by comparing the begging calls of 19–20-day-old New Zealand cuckoos with younger host nestlings. A total of 32 recordings of host nestlings were collected from 23 nests, with each nest containing 1–3 host nestlings: 8 recordings from 7 nests of yellow-rumped thornbills containing chicks aged 3–12 days (AU_host), 18 recordings from 11 nests of fan-tailed gerygone containing chicks aged 3–11 days (NC_host) and 6 recordings from 6 nests of grey warbler containing chicks aged 8–12 days (NZ_host). In Australia, begging calls were recorded for 20 min using a Sony tie-clip miniature microphone (ECM T6) and a digital recorder (TASCAM DR-05). The microphone was clipped to vegetation approximately 20 cm from the nest entrance. In New Caledonia, nestlings were recorded using a digital recorder (Tascam DR-40) and a condenser microphone (Shure SM93), placed at the bottom of the nest 1–3 cm from the internal incubation chamber at multiple feeding events during 2–3 h of recording sessions. In New Zealand, nestling begging calls were recorded using a Sennheiser ME 66 microphone or a Panasonic RP-VC201 stereo tie-clip microphone, depending on nest accessibility, onto a Sony MZ-NH700 Hi-MD Minidisc. Young cuckoo fledglings were recorded using the Sennheiser ME66 directional microphone, while being fed by their host parents outside the nest. We measured only robust spectrogram-based features (see measurements below) from the recordings, therefore it is unlikely that the use of different recorders introduced biases or artefacts in the analyses.

Preliminary visual analysis of the spectrograms (settings: Hann window, 3 dB bandwidth 135 Hz, 90% overlap, hop size 51 samples, DFT 512 samples) showed that cuckoo and host nestlings aged 3–6 days produced short and high-pitched peeps, which then turned into calls with long duration and slow-frequency modulation at the age of 7–10 days and were finally fixed into calls with long duration, loud volume and fast-frequency modulations at the age of 11–14 days. Therefore, our recordings were divided into three age groups, each of 4-day duration: 3–6 days, 7–10 days and 11–14 days. The recordings of 19–20 day-old NZ_parasite in the 11–14 days group were included to allow comparison of this cuckoo subspecies with younger cuckoo and host nestlings in our data set. Audacity (version 3.6) was used to visually scan spectrograms and to determine when parents landed on the nest (marked by a loud thump followed by parental calls) causing a begging response from their nestlings and to export all the begging events in each recording into 30s-long separate audio files. All extracted recordings were normalized to 0 dB, and noise reduction was applied

to eliminate background noise. A call was defined as a single uninterrupted trace on the spectrogram. For each recording session we selected the audio file with the best quality in which calls left a clear trace on the spectrogram and did not overlap with parental calls or other external sound sources. Ten consecutive calls were isolated from the selected audio files starting from the first begging in response to the parents' arrival (with the exception of one recording of a cuckoo nestling from New Caledonia in which only nine consecutive calls were isolated). If the nest contained multiple host nestlings with calls overlapping in time, then a single nestling (identifiable by calls with similar frequency, amplitude and timing) was isolated, and the loudest and clearly visible 10 consecutive calls in response to the parents' arrival were selected. The duration, lowest frequency, highest frequency, frequency bandwidth, centre frequency (frequency dividing the selection in two intervals of equal energy), 5% frequency (frequency at which the total energy of the call exceeds 5%), 95% frequency (frequency at which the total energy of the call exceeds 95%), peak frequency (frequency at which the highest amplitude occurs) and average call entropy (variation of sound intensity across time and frequency, with 0 being a call of constant frequency) from each call in the selected audio files was measured. Raven Pro 1.6.5 (Center for Conservation Bioacoustics, 2023) was used to produce spectrograms and measure call parameters.

Statistics

Linear discriminant analysis (LDA) and multinomial logistic regression (MLR) are commonly used as classification methods to predict the category of an element based on the characteristics of a training data set, thereby estimating the similarities among elements of groups. LDA assumes multivariate normality, whereas MLR can be used as a non-parametric alternative. Nevertheless, the reliability of LDA or MLR is dependent on the sample size and skewness of the outcome variable, with LDA being more reliable with low sample sizes and moderate violations of normality assumptions (Pohar et al., 2004; Jamie et al., 2020). We thus predicted begging call similarities among nestling species using both methods and obtained similar results. Therefore, we report LDA results in the main text and MLR results in the supplementary material (Tables S1–2). LDA was used to quantify the extent of begging call mimicry by each cuckoo subspecies of the respective local host using the function `lda` in R 4.4.1 (R Core Team, 2024). Following the method described by Jamie et al. (2020), all the measured calls from each chick, rather than their average, were used as the raw units for our analysis, and nine measured call parameters were included to maximize the discrimination power of the discriminant function. We aimed to investigate whether the begging calls of a cuckoo subspecies were more similar to the parasitized host than to non-hosts, thus shaped by coevolutionary interactions, or to the cuckoo subspecies from other regions, thus shaped by phylogenetic relatedness. Thus, the full data set was divided in accordance with the age group (3–6, 7–10 and 11–14), and two training subsets containing (1) the calls of the three host species and (2) the calls of the main host of the focal cuckoo subspecies plus the two non-focal cuckoo subspecies from the other regions were used. The two subsets were used to train LDA algorithms for each age group, and the subsets of the calls of the focal cuckoo subspecies (AU_parasite, NC_parasite or NZ_parasite) were used to predict the most likely classification of each call in accordance with the LDA classification function. Additionally, we verified if the begging calls of the two gerygone host species were more similar to each other, thus linked by phylogenetic relatedness, than to the Australian host and the three cuckoo subspecies. To do so, we used two training subsets that included all begging calls from

nestlings of all ages combined without the focal gerygone and used the subsets of the focal gerygone calls (either NC_host or NZ_host) to predict the most likely classification according to the LDA function.

Generalized linear models (GLM) with gamma distribution were used to analyse differences in call duration, lower frequency, higher frequency, frequency bandwidth, peak frequency and average entropy between each cuckoo subspecies and their respective host. The 5%, 95% and centre frequency were excluded from GLM analysis because our aim was to detect the most significant differences in the higher order and more informative call parameters between hosts and parasites. The average values of the focal call parameter for each individual were used as outcome variable, and the species, age and their interaction were used as response variables. An interaction comparing the New Zealand host and parasite was not included as our data included only one age group (11–14 days) for the New Zealand parasite. The models were validated by checking for the normality of model residuals and model fit. The function Anova from the package car was used to extract ANOVA tables for each GLM model.

Finally, the call rate (in calls/s) was compared among species and age groups. The call rate was log transformed to achieve a normal distribution, and linear regression (function lm in R) was used to test the relationship of call rate with call duration. Then, two-way ANOVA (function aov in R) was performed with call rate as outcome variable and species, age group and their interaction as response variables. The two-way ANOVA indicated the significant effect of species (check Results). Therefore, a Tukey post hoc test (function TukeyHSD in R) was used to identify significant differences in pairwise comparisons between species (Supplementary Material Table S4).

Ethical Note

The recordings were collected by placing a microphone outside the nest while parents were away during their foraging bouts, and the nestlings were not manipulated. The microphone was installed in less than 1 min; therefore, the disturbance of the breeding was kept at a minimum. None of the monitored breeding attempts failed as a result of the recording procedure. The research in New Caledonia was conducted under permit numbers 2476–2015, 2372–2017, 3469–2018, 2720–2019, 1679–2023 and 5818–2023 issued by Province Sud (New Caledonia), and ethical approval was not required. Research in New Zealand was conducted with approval by the Massey University Animal Ethics Committee. The research in Australia was conducted with approval by Environment ACT (licence numbers LT2005164, LT2006229, LT2007266 and LT2009378) and the Australian National University Animal Experimentation Ethics Committee (permit numbers F.BTZ.00.06, F.BTZ.61.03 and F.BTZ.39.09).

RESULTS

The discriminant function classified the begging calls of each cuckoo subspecies in all age groups as being more similar to the parasitized host than to the hosts from other regions, thereby showing that the cuckoo calls closely matched the host across the whole nestling period (Table 1; Fig. 1). The spectrograms of the begging calls showed a resemblance of the call structure between each cuckoo subspecies and the respective parasitized hosts, thereby supporting the high similarity scores of the discriminant analysis (Supplementary material, Figs. S1–3). The begging calls of each cuckoo subspecies were often classified as being more similar to the phylogenetically unrelated main host than to the begging

Table 1

Predicted similarity of begging calls in accordance with the linear discriminant function for each of the cuckoo–host pairs from each region

	AU_host	NC_host	NZ_host
Age 3–6 days			
AU_parasite	47	23	–
NC_parasite	27	72	–
Age 7–10 days			
AU_parasite	13	0	7
NC_parasite	0	54	6
Age 11–14 days			
AU_parasite	10	0	0
NC_parasite	0	20	10
NZ_parasite	4	5	31

Values in boldface indicate the natural cuckoo–host pair occurring in each region. AU: Australia; NC: New Caledonia; NZ: New Zealand. NZ_parasite cuckoos from New Zealand are 19–20 days old.

calls of cuckoo subspecies from other regions, but the discrimination function returned relatively high similarity scores of begging calls from the two cuckoo subspecies from New Caledonia and New Zealand (Table 2).

The frequency and entropy of the calls explained the main differences between cuckoos and hosts from Australia and New Caledonia, whereas only differences in the entropy of the calls were observed in New Zealand (Table 3, Fig. 2). In addition, age-related differences in call duration were found in Australia and New Caledonia, with the cuckoo–host pair from New Caledonia showing pronounced differences in the high frequency and entropy of the calls, whereas the cuckoo–host pair from New Zealand showed no major age-related differences (Table 3). Overall, the cuckoo–host pair from New Caledonia had the largest difference between species and age classes, whereas the cuckoo–host pair from New Zealand was the most similar compared with that from the other two regions (Table 3; Fig. 2).

Across all ages, the begging calls of the host from New Caledonia ($N = 180$) had high similarity scores with the respective brood parasite (64%), relatively high similarity to the closely related host from New Zealand (34%) and low similarity to the New Zealand cuckoo subspecies (2%; Supplementary material, Table S3). On the contrary, the calls of the host from New Zealand ($N = 60$) were mostly similar to the calls of the closely related host from New Caledonia (95%) than to the respective brood parasite (5%), and they showed no similarity to the cuckoo subspecies from New Caledonia (Supplementary material, Table S3). The hosts from New Caledonia and New Zealand showed no similarity to the Australian host and cuckoo subspecies (Supplementary material, Table S3). A visual inspection of the spectrograms showed high similarity to the begging calls of gerygone chicks from New Caledonia and New Zealand (Supplementary material, Figs. S2–3), thereby supporting the prediction scores of the discriminant function and a similarity of begging calls caused by phylogenetic relatedness of these two host species.

Call rate was related to call duration, with longer calls leading to a slower rate (linear model; $F_{1,63} = 14.52$, $P < 0.001$), but it did not depend on nestling age (ANOVA; $F_{2,50} = 2.089$, $P = 0.134$). Species had an effect on call rate (ANOVA, $F_{5,50} = 3.841$, $P = 0.005$). However, a post-hoc comparison revealed that this effect was not due to the differences within a natural cuckoo–host pair from the same region, but rather due to differences between AU_host and nestlings from other regions (AU_host versus NC_parasite, $P = 0.008$; AU_host versus NZ_parasite, $P = 0.048$; AU_host versus NZ_host, $P = 0.014$; Supplementary material, Fig. S4; Table S4).

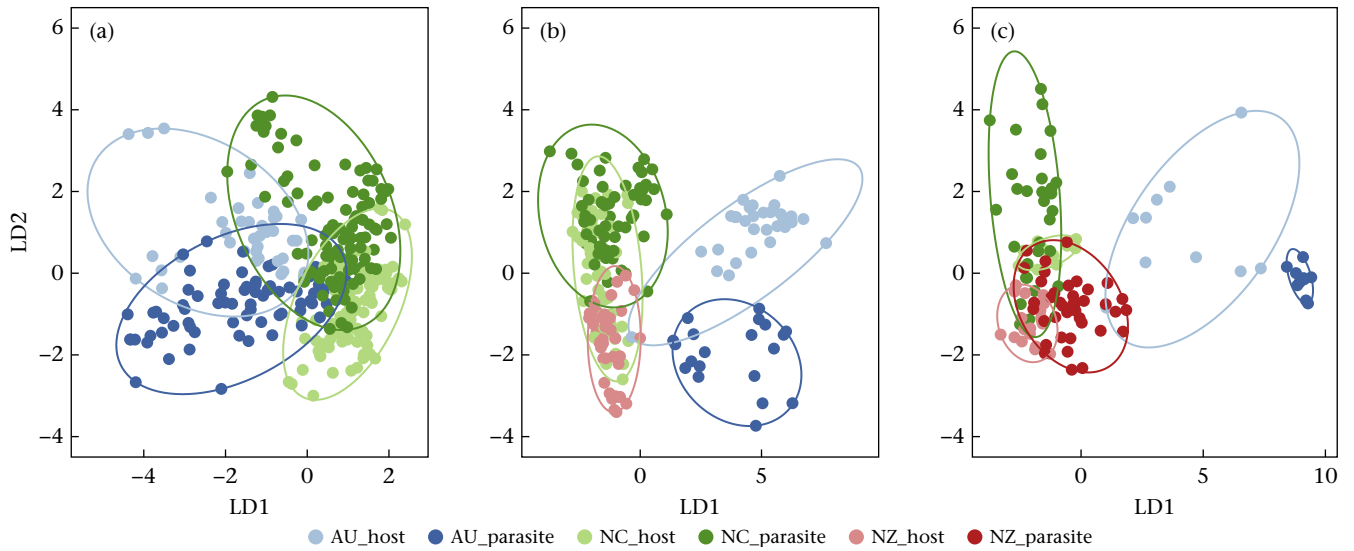


Figure 1. Linear discriminant biplots for begging calls of chicks aged (a) 3–6 days, (b) 7–10 days and (c) 11–14 days. Shining bronze-cuckoos from New Zealand aged 19–20 days are included in the day 11–14 group. Labels indicate the region. AU: Australia; NC: New Caledonia; NZ: New Zealand. Ellipses indicate hulls surrounding all nestlings within each species, with smaller distances indicating higher similarities between groups.

DISCUSSION

The begging calls of nestlings of each shining bronze cuckoo subspecies match closely those of their respective hosts. However, none of the begging calls of cuckoo subspecies is a perfect match of the host, and the species-specific differences in the frequency and entropy of the begging calls might allow the host to discriminate the parasite nestling. Such differences are more pronounced in the cuckoo–host pair from New Caledonia, indicating a less accurate matching of the host in the cuckoo subspecies from New Caledonia compared with the other subspecies. This might explain why the host from New Caledonia rejects the cuckoo nestlings, whereas the hosts from Australia and New Zealand do not. As expected, the begging calls of the two gerygone hosts from New Caledonia and New Zealand are more similar to each other than to the thornbill host from Australia because of phylogenetic relatedness. Similarly, the begging calls of the two cuckoo subspecies from New Caledonia and New Zealand are more similar to each other than to the cuckoo subspecies from Australia, thereby indicating convergence in begging call features caused by parasitism of two closely related

hosts. Overall, the results support our hypothesis that the structure of the begging calls of each shining bronze-cuckoo subspecies is mainly shaped by coevolutionary interactions with their hosts rather than phylogeny.

Discrimination of parasite nestlings by the host is a major factor driving the selection for matching of the host begging calls by the parasite (Jamie & Kilner, 2017). However, some factors may contribute to the similarity in the structure of begging calls between parasites and hosts. For example, phylogenetic relationships might constrain variation in begging call features, thereby resulting in a similar call structure in parasites and hosts, or the similarity could result from random matching between the parasite and the host (Grim, 2005). However, the shining bronze-cuckoo and its hosts are not closely related, thus it is highly unlikely that the call matching is ancestral. Moreover, the pattern of similarity is not random as the nestlings of the three shining bronze-cuckoo subspecies closely match the nestlings of the respective host visually and acoustically (Attisano et al., 2023). Parallel evolution caused by shared ecological factors might also result in the matching of begging call features, for example, nest predation might influence the frequency and amplitude of the calls (Briskie et al., 1999) and lead to a partial similarity of the begging calls between the parasite and the host (Grim, 2005). However, the three hosts live in highly different habitats, and they are exposed to different main predators: birds in New Caledonia (Attisano et al., 2020); reptiles, birds and mammals in Australia (Guppy et al., 2017) and introduced mammals in New Zealand (Remeš et al., 2012). Thus, selection by nest predators does not explain the matching of multiple call features of the hosts by the three shining bronze-cuckoo subspecies across their range. Therefore, call matching of the host by the shining bronze-cuckoo subspecies is likely explained by mimicry resulting from coevolutionary interactions driven by host discrimination (Dalziell & Welbergen, 2016; Jamie & Kilner, 2017), which is consistent with the highly accurate visual mimicry of host nestlings by the three cuckoo subspecies.

This finding is exemplified by the cuckoo–host pair from New Caledonia. The fan-tailed gerygone host removes 90% of the parasite nestlings within 24 h from hatching using visual features and hatching order to discriminate parasite nestling (Attisano et al., 2018, 2021). A closely related rejector host from Australia, the

Table 2
Predicted similarity of begging calls in accordance with the linear discriminant function for each of the three cuckoo subspecies compared with the local main host and the other cuckoo subspecies from other regions

	Main host	AU_parasite	NC_parasite	NZ_parasite
Age 3–6 days				
AU_parasite	52	–	18	–
NC_parasite	62	37	–	–
Age 7–10 days				
AU_parasite	20	–	0	–
NC_parasite	55	5	–	–
Age 11–14 days				
AU_parasite	10	–	0	0
NC_parasite	15	0	–	15
NZ_parasite	8	0	32	–

Values in boldface indicate the pair combination with the highest predicted similarity. AU: Australia; NC: New Caledonia; NZ: New Zealand. NZ_parasite cuckoos from New Zealand are 19–20 days old.

Table 3
Results of the generalized linear models for the comparison of begging call features in cuckoo–host pairs from Australia, New Caledonia and New Zealand

	Species			Age			Species:Age		
	χ^2	df	P	χ^2	df	P	χ^2	df	P
<i>C. l. plagosus</i> vs <i>A. chrysorroa</i> (Australia)									
Duration	0.259	1	0.611	12.362	2	0.002	3.157	2	0.206
Lowest frequency	10.350	1	0.001	1.949	2	0.377	0.893	2	0.640
Highest frequency	8.954	1	0.003	1.477	2	0.478	0.070	2	0.965
Frequency bandwidth	1.618	1	0.203	1.115	2	0.573	0.324	2	0.850
Peak frequency	6.721	1	0.010	1.480	2	0.477	0.915	2	0.633
Average entropy	2.019	1	0.155	3.846	2	0.146	2.563	2	0.278
<i>C. l. layardi</i> vs <i>G. flavolateralis</i> (New Caledonia)									
Duration	0.056	1	0.813	13.084	2	0.001	0.098	2	0.952
Lowest frequency	13.623	1	<0.001	5.717	2	0.057	0.146	2	0.930
Highest frequency	0.803	1	0.370	6.582	2	0.037	1.358	2	0.507
Frequency bandwidth	13.496	1	<0.001	1.530	2	0.465	2.972	2	0.226
Peak frequency	1.563	1	0.211	3.937	2	0.140	0.492	2	0.782
Average entropy	29.139	1	<0.001	15.747	2	<0.001	1.896	2	0.387
<i>C. l. lucidus</i> vs <i>G. igata</i> (New Zealand)									
Duration	<0.001	1	0.991	1.235	1	0.266	–	–	–
Lowest frequency	1.292	1	0.256	1.912	1	0.167	–	–	–
Highest frequency	1.216	1	0.270	1.183	1	0.277	–	–	–
Frequency bandwidth	3.139	1	0.076	0.132	1	0.716	–	–	–
Peak frequency	0.010	1	0.919	1.222	1	0.269	–	–	–
Average entropy	9.969	1	0.002	0.748	1	0.387	–	–	–

Values in boldface indicates significant differences ($\alpha < 0.05$).

large-billed gerygone *G. magnirostris*, is able to discriminate about 69% of the nestlings of the little bronze-cuckoo via visual features (Noh et al., 2018) and acoustic differences in begging calls (Noh et al., 2021). The variation in rejection rates between the two gerygone hosts can be explained by the accuracy of visual and auditory matching of the host by the parasite. The New Caledonian shining bronze-cuckoo is indeed a less accurate match of its host compared to the Australian little bronze-cuckoo (Noh et al., 2018, 2021; Sato et al., 2010). This would explain the high rejection rates of the parasite and absence of recognition errors in the fan-tailed gerygone (Attisano et al., 2018, 2021) compared to the lower rejection rates of the large-billed gerygone and the fact that this host sometimes reject its own nestlings by mistake (Noh et al., 2018, 2021; Sato et al., 2010). The results of this study suggest that, apart from the visual features of the nestlings, the fan-tailed gerygone might use acoustic differences in the structure of the begging calls to discriminate parasite nestling. This process has led to selection for mimicry of the host begging call by the New Caledonian shining bronze-cuckoo to escape host discrimination.

The grey warbler and yellow-rumped thornbill hosts do not have nestling discrimination ability as they have not been observed rejecting shining bronze-cuckoo nestlings either by ejection from the nest or by nest abandonment (Medina & Langmore, 2016; Thorogood et al., 2017). The matching of the host begging calls by the shining bronze-cuckoo nestlings in these systems might result from parasite tuning into the offspring–parent communication system of the host to manipulate parental provisioning (Davies, 2011). In this case, nestling discrimination by the host might not be a necessary condition to select for begging call mimicry (Grim, 2005), and the matching could result from the parasite nestling producing calls that maximize the chances of obtaining food from the host parents (Langmore et al., 2008). Other brood parasites signal their hunger state to the host parents with exaggerated begging traits such as a fast call rate (Kilner et al., 1999; Roldán et al., 2013; Tuero et al., 2016) or postural components of the begging display (Jamie et al., 2020), which induce host parents to increase feeding rate, thereby providing an adequate amount of food to the demanding parasite chick (Soler, 2017b). The postural components of the begging display were not quantified in this

study, but we did not find evidence of exaggerated traits such as a faster call rate in any of the shining bronze-cuckoo subspecies as they all matched the begging rate of individual host chicks across the range. The grey warbler and yellow-rumped thornbill hosts might have a preference for chicks begging at the 'right' rate, that is the rate of a single host chick, rather than an exaggerated one mimicking a whole brood (Davies et al., 1998). This phenomenon could depend on a limit to the amount of food that the small hosts of the shining bronze-cuckoo can provide as they all have relatively small brood sizes of one to three chicks and therefore might not be able to increase their provisioning rate in response to exaggerated begging displays of the cuckoo.

Each of the three cuckoo subspecies visually matches their respective host in each region (Attisano et al., 2023). Therefore, the matching of the host begging calls supports a scenario of selection for multimodal mimicry of the hosts by the shining bronze-cuckoo. The shining bronze-cuckoo from Australia matches visual and acoustic features of its host and the visual mimicry is the most accurate among the three cuckoo subspecies (Attisano et al., 2023; this study). In New Zealand, phylogenetic comparative analysis shows that the local shining bronze-cuckoo subspecies matches the begging calls of the grey warbler host more closely than any other New Zealand passerine species (Anderson et al., 2009; McLean & Waas, 1987; Ranjard et al., 2010), and in this study we found that the acoustic mimicry of the host is the most accurate among the three cuckoo subspecies. Although only the fan-tailed gerygone host from New Caledonia currently discriminates the cuckoo nestling, the evidence suggests that the grey warbler from New Zealand and the yellow-rumped thornbill from Australia might have been able to discriminate parasite nestlings at least at some point in the past, most likely in the early stages of the coevolutionary arms race with the shining bronze-cuckoo. Nowadays these hosts might no longer accurately discriminate the parasite due to the refined level of mimicry achieved by the shining bronze-cuckoo nestlings. The presence of moderately effective anti-parasitic defences at the nest-building and egg stages in both these hosts (Anderson et al., 2013; Medina & Langmore, 2016), might further relax selection for improved defences at later stages of the arms race (Britton et al., 2007) and as a consequence the nestling discrimination behaviour

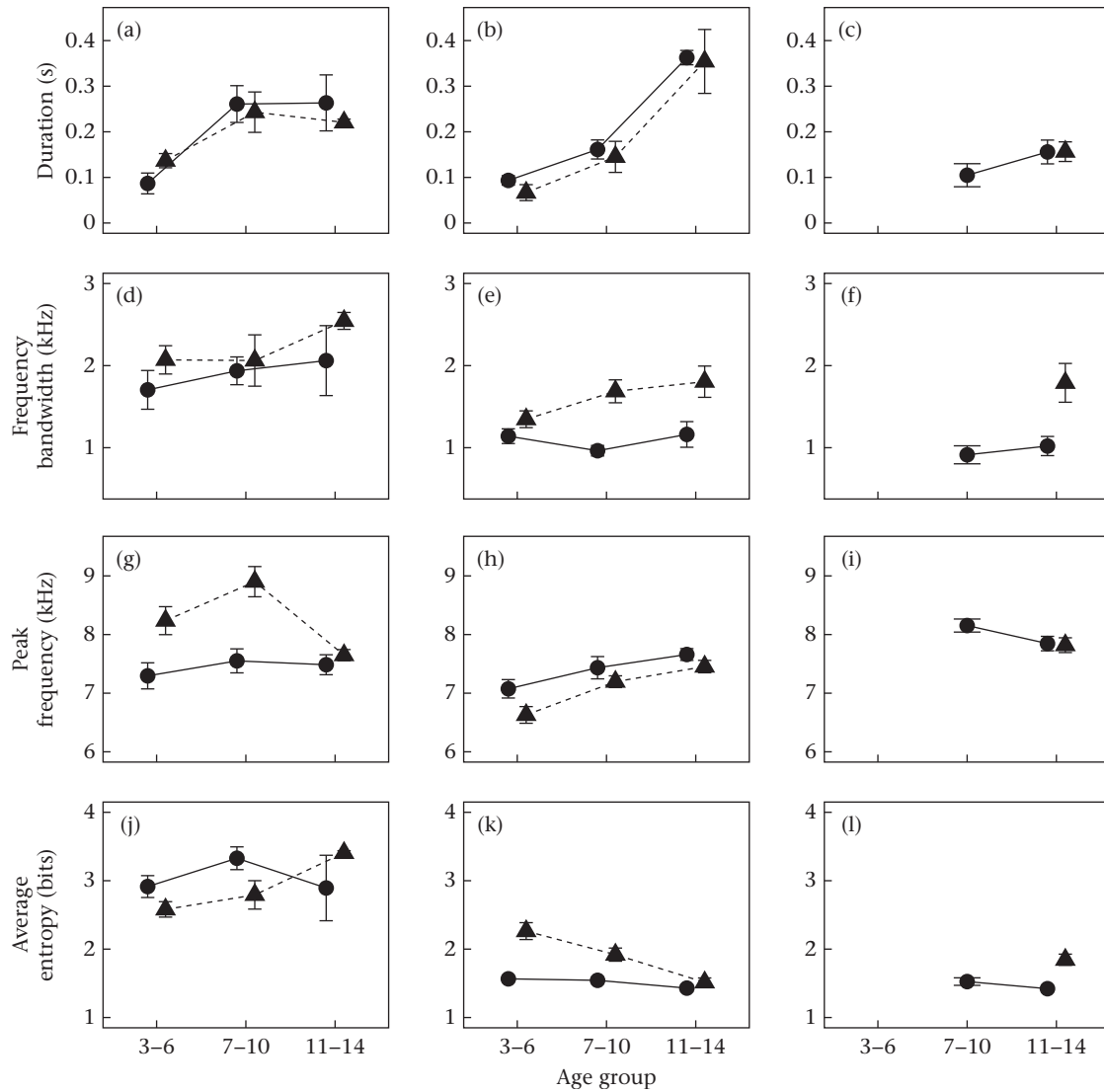


Figure 2. Duration, frequency bandwidth, peak frequency and average entropy of begging calls of cuckoo (triangles) and host (circles) pairs from (a, d, g, j) Australia, (b, e, h, k) New Caledonia and (c, f, i, l) New Zealand. Error bars represent 95% confidence intervals.

has been lost or reached frequencies too low to be detected during field monitoring.

In conclusion, we found support for our hypothesis that the structure of the begging calls of each shining bronze-cuckoo subspecies is shaped primarily by coevolutionary interactions, and that the calls of each subspecies has been altered to match those of the specific host in their area of distribution. The selection for a refined multimodal mimicry of the host via visual and acoustic signals has promoted phenotypic differentiation of the parasite nestlings across the distribution range with the formation of geographically and genetically separated host races (Gill, 1998; Noske, 2019), which are adapted to exploit the specific local host.

Author Contributions

Alfredo Attisano: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Michael G. Anderson:** Writing – review & editing, Investigation, Funding acquisition, Data curation. **Naomi E. Langmore:** Writing –

review & editing, Investigation, Funding acquisition, Data curation. **Roman Gula:** Writing – review & editing, Funding acquisition. **Jörn Theuerkauf:** Writing – review & editing, Funding acquisition, Conceptualization.

Data Availability

Data, R code and additional analyses are available as supplementary material.

Declaration of Interest

The authors declare that they have no conflict of interest.

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Supplementary Material

Supplementary material associated with this article is available at <https://doi.org/10.1016/j.anbehav.2025.123083>.

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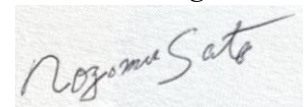
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1.1. Sato, N.J.

I declare that my contribution to the above article consisted in formulating the main research hypothesis, obtaining funding, collecting data in the field and revising the manuscript.

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2. Attisano, A., Hlebowicz, K., Gula, R., & Theuerkauf, J. (2021). Threat recognition and response in an avian brood-parasite host from New Caledonia. *Current Zoology*, 67: 255–262. <https://doi.org/10.1093/cz/zoaa061> [IF₂₀₂₁ = 2.734 ; Q1; MNiSW points = 100]

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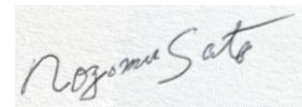
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3. Attisano, A., Sato, N. J., Tanaka, K. D., Okahisa, Y., Ueda, K., Gula, R., & Theuerkauf, J. (2021). Discrimination and ejection of eggs and nestlings by the fan-tailed gerygone from New Caledonia. *Current Zoology*, 67: 653–663. <https://doi.org/10.1093/cz/zoab066> [IF₂₀₂₁ = 2.734; Q1; MNiSW points = 100]

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3.2. Tanaka, K.D.

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3.3. Okahisa, Y.

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3.5. Gula, R.

I declare that my contribution to the above article consisted in obtaining funding and revising the manuscript.


Signature

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3.6. Theuerkauf, J.

I declare that my contribution to the above article consisted in formulating the main research hypothesis, obtaining funding and revising the manuscript.

Signature

A handwritten signature in black ink, appearing to read 'J. Theuerkauf', written on a white rectangular background.

4. Attisano, A., Gill, B.J., Anderson, M.G., Gula, R., Langmore, N.E., Okahisa, Y., Sato, N.J., Tanaka, K., Thorogood, R., Ueda, K., Theuerkauf, J. (2023). Polymorphism at the nestling stage and host-specific mimicry in an Australasian cuckoo-host arms race. *Journal of Animal Ecology* 93: 30-43, <https://doi.org/10.1111/1365-2656.13849> [IF₂₀₂₃ = 3.5 ; Q1; MNiSW points = 140]

4.1. Gill, B.J.

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4.2. Anderson, M.G.

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4.3. Gula, R.

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4.4. Langmore, N.E.

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4.5. Okahisa, Y.

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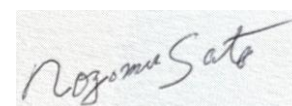
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4.6. Sato, N.J.

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4.7. Tanaka, K.D.

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4.8. Thorogood, R.

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4.9. Ueda, K.

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4.10. Theuerkauf, J.

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Signature

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5. Attisano, A., Anderson M.G., Langmore N.E., Gula, R., Theuerkauf J. (2025). Begging call mimicry and formation of host-specific lineages in the shining bronze-cuckoo *Chalcites lucidus*. *Animal Behaviour* 221:123083, <https://doi.org/10.1016/j.anbehav.2025.123083> [IF₂₀₂₅ = 2.3 ; Q1; MNiSW points = 140]

5.1. Anderson, M.G.

I declare that my contribution to the above article consisted in obtaining funding, collecting data from New Zealand and revising the manuscript.

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5.2. Langmore, N.E.

I declare that my contribution to the above article consisted in obtaining funding, collecting data from Australia and revising the manuscript.

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5.3. Gula, R.

I declare that my contribution to the above article consisted in obtaining funding and revising the manuscript.

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5.4. Theuerkauf, J.

I declare that my contribution to the above article consisted in obtaining funding and revising the manuscript.

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