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Author for correspondence:
Thomas N. Sherratt
e-mail: tom.sherratt@carleton.ca

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The anti-predation benefit of flash displays is related to the distance at which the prey initiates its escape

Karl Loeffler-Henry[1], Changku Kang[2] and Thomas N. Sherratt[1]

[1]Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6
[2]Department of Biosciences, Mokpo National University, Muan, Jeollanamdo 58554, South Korea

(iD) CK, 0000-0003-3707-4989; TNS, 0000-0001-6837-3848

Flash behaviour is widespread in the animal kingdom and describes the exposure of a hidden conspicuous signal as an animal flees from predators. Recent studies have demonstrated that the signal can enhance survivorship by leading pursuing predators into assuming the flasher is also conspicuous at rest. Naturally, this illusion will work best if potential predators are ignorant of the flasher's resting appearance, which could be achieved if the prey flees while the predator is relatively far away. To test this hypothesis, we compared the survival of flashing and non-flashing computer-generated prey with different flight initiation distances (FIDs) using humans as model predators. This experiment found that flash displays confer a survivorship advantage only to those prey with a long FID. A complementary phylogenetic analysis of Australian bird species supports these results: after controlling for body size, species with putative flashing signals had longer FIDs than those without. Species with putative flashing signals also tended to be larger, as demonstrated in other taxa. The anti-predation benefit of flash displays is therefore related to the nature of escape behaviour. Since birds with hidden signals tend to flee at a distance, the flash display here is unlikely to function by startling would-be predators.

1. Introduction

Many species have evolved colour patterns that resemble their backgrounds to avoid being detected by predators [1]. However, even cryptic species occasionally need to move, and this motion increases the chance that they will be seen and/or heard by predators [2]. One way in which a cryptic species may mitigate their increased detection risk during motion is through the adoption of a flash display. Flash displays involve the sudden exposure of a previously hidden conspicuous signal when the animal is moving, followed by its concealment when the movement ceases [3,4]. Putative examples of flash behaviour include the brightly coloured hindwing displays seen in many insect groups (notably Orthoptera and Lepidoptera [5]), the tail flagging behaviour of some Artiodactyla and Leporidae [6], and the conspicuous rump and underwing coverlets of many otherwise cryptic bird species, all of which are revealed only in flight [3,4]. The combination of exposing a hidden signal and movement distinguishes flash behaviour from deimatic, (startle) displays which typically involve exposing previously hidden signals while stationary [7].

While flash behaviour may serve to surprise any would-be predator [4] and make the flasher harder to catch while fleeing [8], an important benefit is that would-be predators are misled into anticipating the prey to be always conspicuous in appearance, when it is not [3]. Specifically, if a predator encounters a conspicuous prey item that is in the process of fleeing, the predator may continue to search for the prey, assuming the prey retains the same conspicuous appearance on settling, when in fact it has reverted to its cryptic state. Supposing the prey is no longer present, the predator may more readily give up its search. In support, a computer-based experiment using humans as visual

predators found that participants were indeed more likely to give up looking for prey that displayed conspicuous colours when in motion but cryptic colours when at rest, compared to those prey that had cryptic coloration both in motion and at rest [9].

Despite their widespread taxonomic distribution [3,5], flash displays have only recently begun to be investigated and relatively little is known about the factors that mediate their effectiveness. Several comparative studies have reported that putative flash displays are more likely to have evolved in larger species [5,10]. Motivated by these observations, a recent experiment with computer-generated prey demonstrated that flash behaviour resulted in a higher survival benefit in large prey compared to small prey, in part because smaller prey have high survivorship even without a flashing signal, leaving less room for an improvement [11]. Here we address another, perhaps more important, condition that may mediate the effectiveness of flash signals, namely the distance at which the signallers initiate their escape from an approaching predator.

When faced with an approaching predator, prey have to decide whether, and when, to flee [12]. If flashing prey do indeed gain an anti-predator benefit from misleading the predator into supposing they are always conspicuous, then the success of this illusion might depend on whether the predator has observed the prey in its resting state before disturbance. Naturally, if prey wait until a predator closely approaches, then the predator will have more opportunity to detect the prey and directly observe its transition from crypsis to conspicuousness. Under these conditions, the effectiveness of the flash display is likely to be significantly reduced. In this study, we began by testing whether the distance from a predator at which a flashing prey starts to flee (and hence the degree of opportunity of predators to see them in their cryptic state) affects the effectiveness of the flash display in reducing subsequent detection.

The distance at which an animal initiates escape from a predator in the wild has been variously referred to as a 'flight initiation distance' (FID), 'escape initiation distance' or 'flush distance'. FIDs have been widely reported in the literature and found to vary across species within many taxonomic groups [13–17], at least in part as a consequence of interspecific variation in predation risk, with species at higher predation risk tending to have longer FIDs [18–20]. Some consistent covariates with FID within and among species have, therefore, been identified (e.g. [21]). Of particular relevance to this study, body mass explains considerable variation in FID among species, especially in birds with larger species having a longer mean FID [22]. Although the underlying reasons for this association are unclear, large species are potentially more vulnerable in that they may be more detectable from a distance, less agile and/or more profitable to pursue [23].

Having experimentally evaluated how the efficacy of flash displays varies with FID, we next determined whether the presence of hidden conspicuous signals is associated with FID in birds through phylogenetic comparative analysis. Birds are an excellent group to investigate the relationship between flash displays and the FID because FID has been carefully documented in many bird species under standardized conditions [24]. Although flash displays in birds have not yet been systematically evaluated, many species have conspicuous patches that are only visible when they are flying, which putatively function as a flashing signal. As noted

above, we predict that species with putative flashing signals would initiate their escape from a greater distance than the species without such signals since the illusion is likely to be more effective when would-be predators do not see their resting state.

2. Methods

(a) Human predator experiment

To elucidate the relationship between FID and the efficacy of flash displays, a computer experiment was conducted from February to March 2019 (see electronic supplementary material for a demonstration video). The experiment involved 30 volunteers playing a simple computer game that was modified from Loeffler-Henry et al. [9]. Participants (largely undergraduates) were recruited as they were entering and leaving Carleton University MacOdrum library, Ottawa, Canada. The game was developed in Microsoft Visual Basic 6 and displayed on a Toshiba Portégé laptop. Before the experiment began, we asked the volunteers to read scripted instructions explaining how to play the game. The details of our hypothesis were not disclosed to our participants. When the participant indicated that they had understood the nature of the game and were ready to play, the game started.

Each game comprised 12 trials, with square artificial prey (400×400 twips; 15 twips = 1 pixel) presented against the same complex grass background (dimensions 12 700 twips height $\times$ 8170 twips width). Each trial had a fleeing stage followed by a settling stage. At the beginning of the fleeing stage, a prey was placed at a random location on the grass background. When participants moved the mouse pointer within a specified reactive distance (corresponding to a FID) of the prey item, the prey item moved to either right or left (whichever side was further) in a directed random walk off the screen. This allowed the participants to see the fleeing prey for 1–2 s on the screen. After the prey item disappeared from sight, participants were invited to press a 'follow' button which led to a new screen containing a mirror image of the previous background, often (but not always; see below) containing the same prey. The background was mirrored in this settling stage screen to prevent our volunteers locating the prey by contrasting an otherwise identical background image. On following the prey to the new area, our volunteers had two options: they could either capture the prey by moving their mouse pointer over it and clicking on it or, if they failed to find it and considered the prey were not present on the screen, they could press a 'give-up looking' button. When participants found the prey or pressed the give-up looking button, they were then presented with a 'next prey' button which led them to the next trial (or the game was ended if this was the last prey to be presented).

The first eight trials were used as the training phase to acclimate the participants to the game, while the last four trials were used for testing the efficacy of flash displays. The training phase and testing phases were essentially the same, except that flashing prey (with conspicuous colours when they were moving yet cryptic colours when they settled) were presented only in the test phase. During the training phase, participants were exposed to eight prey of different colours (all readily detectable due to their high contrast against the grass background; see RGB values in electronic supplementary material, table S1) presented in random order. The colours of the prey were fixed throughout the fleeing and settling stage. The FID of the training prey that prompted movement was the mouse moving within 500 twips of the centre of the prey (1.25 times prey lengths and approximately 6% of the background width), allowing all participants an opportunity to see the prey's resting colours before it moved. Five of the training prey (blue, yellow, cyan, magenta

and green) were present both in the fleeing and settling stages. The remaining three training prey (set as blue, magenta and yellow) were only present in the fleeing stage but not in the settling stage, ultimately forcing participants to press the 'give-up looking' button and move on to the next screen. We included these 'dud' trials, so that volunteers would get used to the possibility that sometimes a given prey item would not be present in the search area, and the volunteer would therefore not continue to search the background indefinitely [9]. This mimics the natural situation where a predator follows an escaping prey but searches in the wrong area where the prey is not present.

After the training phase, the testing phase began in which four treatment prey were presented in random order to each participant. These treatment prey followed a 2 × 2 factorial combination of 'flashers' (cryptic when sedentary but red in motion during the fleeing stage) and 'non-flashers' (always with cryptic coloration) with short or long FIDs. Each participant was presented with all four treatment prey types. However, each participant only saw each treatment once and in a random order (first–fourth). All treatment prey in the testing phase were visible in the settling stage, so there were no 'duds'. The cryptic colour of prey had the R, G and B values of the mean grass background (R = 73, G = 151, B = 19). In the short FID treatment, fleeing was initiated when the mouse pointer was within 500 twips of the prey (1.25 times prey length), as in the training phase. Under these conditions, it was highly likely that the predator would see the prey in its cryptic state before the prey moved. In the long FID treatment, prey initiated fleeing immediately after any movement of the mouse pointer (when prey were within a nominal 50 000 twips of the mouse pointer, which was always the case whenever the new screen appeared). With their hands always on the mouse and viewing where they had just clicked to generate a new screen, our volunteer predators under this treatment had little opportunity to observe the prey item in its resting state before it fled.

(b) Statistical analysis of experimental data

All analyses were conducted in R v. 4.0.3 [25]. We first fitted a generalized linear mixed model to elucidate if and how the presence/absence of flashing signals, the prey's short/long FID (both treated as fixed factors), their order of presentation (treated as a covariate given the approximate logit linearity and lower Akaike information criterion) and volunteer ID (random intercept) explain variation in whether a prey item in the testing phase was ultimately attacked or not (binomial response). We included an interaction term between the presence of flashing signals and the FID in our logistic model because we predicted that the efficacy of flashing signals in reducing predation would depend on FID. Model fitting was conducted using the 'glmer' function (assuming binomial error structure) of the 'lme4' package [26]. The importance of predictors was evaluated by comparing models with and without specific terms using log-likelihood ratio tests (LRT). Following evidence for a significant interaction between the use of hidden signals and the prey's FID, the proportions of the four different prey types attacked (two flash/non-flash prey types with two FIDs) were compared directly. To make such multiple unplanned comparisons while controlling for the type I error rate, Tukey's HSD tests were conducted using the 'glht' function of the 'multcomp' package [27].

To test whether the four treatment prey types differed in their time to attack in the settling stage, while allowing for the fact that some prey were not attacked (i.e. they were right censused at the time of giving up), we conducted a survival (time-to-event) analysis. Specifically, we fitted a mixed-effect Cox proportional hazards model using 'coxme' package [28]. Using time to attack as the response variable (including whether it was censused by giving up), the model structure was the same as above

with the presence/absence of flash signals, the prey's FID and their interaction as the primary factors of interest, the order of presentation as a covariate and volunteer ID as a random factor.

(c) Phylogenetic analysis
(i) Flight initiation distance and body size data acquisition
The FID data for 63 Australian birds were taken from an earlier comprehensive review [24]. These data were originally collected by having a human observer approach birds according to a standardized protocol (explained briefly below). Given the propensity of individual birds to habituate to disturbance [29], the FID data were not collected in areas with frequent human traffic. Birds were located with binoculars or the naked eye and approached by researchers at a standard speed of one pace per second. The FID estimate for each species was based on the mean FID from at least 25 separate individuals of that species, with an initial approach started at a range of distances. Although the initial distance of approach may explain some variation in FID [24], there were no statistically significant differences in population mean starting distances among species, so that each species mean reflects data gathered at a comparable array of start distances. Bird body mass data were obtained from [30].

(ii) Classification of the presence of flash behaviour
We collected three to five images of each bird species under different viewing angles, obtained from Google image searches, restricting ourselves to images from websites in which the birds were identified using scientific names, and which appeared authoritative (based on our own experience with bird identification). Our most commonly used source was [31], but a full list of our sources can be found in our electronic supplementary material. Based on these images, each species was classified as to whether it had putative flashing signals. To generate the classification, we asked 30 independent assessors (undergraduate student volunteers) to classify each species based on the images provided. These assessors had no prior knowledge either of the birds' FIDs or of the hypothesis being tested. Before beginning their classification, the assessors were presented with instruction slides (see electronic supplementary material, figure S1) based on a classification of a series of images of non-Australian birds. We then asked assessors to continue to categorize our Australian bird species as having, or not having, novel colours only visible when in flight (i.e. colours that are not present in their resting appearance as an indication of flash behaviour). For those bird species believed to be sexually dimorphic (nine bird species based on their species descriptions [32]), photos of both males and females were separately presented to the assessors. Despite variation in appearance, both sexes of the sexually dimorphic species were consistently classified in the same way as to whether or not they exhibited hidden signals (five of the nine dimorphic species were classed as having hidden signals; minimum agreement for a given sex/species across partcipants was 73.3%). Since the FID data [24] we had for each bird species was not sex-specific and both sexes of sexually dimorphic species were classified in the same way, then these data were retained in our analysis. Assessment took place over Zoom in 30 separate one-on-one sessions with volunteers in October 2020. To minimize and control for any effect that presentation order may have on the assessor's classification, we divided the assessors into three blocks with three separate random presentation orders of the species' images. Assessors were alternately assigned to one of the blocks based on the order in which the trials took place.

(iii) Phylogenetical analysis
We performed phylogenetically controlled analysis using both (i) the maximum clade credibility (MCC) tree (tree provided

in electronic supplementary material, figure S2) and (ii) 1000 phylogenetic trees randomly sampled from the posterior distribution of a supertree obtained from birdtree.org using the Ericson backbone to account for the uncertainties in topology and branch length [33]. To elucidate the relationship between the mean adult body mass, the presence of putative flashing signals and the mean FID of birds after controlling for their phylogeny, we fitted phylogenetic generalized linear squares (PGLS) models using all trees. In all cases, we set the mean FID of each species as the response variable. For explanatory variables, we used the presence/absence of putative flashing signals (either a binary or continuous predictor, see below for how we created this variable), the mean mass of each species and the interaction between these two. Both the mean FID and mean mass were log-transformed to remove their left-skewness in distributions. We assumed a Brownian motion (BM) model of trait evolution [34]. Alternative models of trait evolution (specifically Pagel's λ and Ornstein–Uhlenbeck models [35]) are known to work poorly in small trees (less than 200 species) and failed to estimate exact parameters in some of the trees [36]. Nevertheless, we compared the estimated parameters and the results of PGLS among different models (BM, Ornstein–Uhlenbeck and Pagel's λ) using subsets of trees and found that the inference did not differ, regardless of which models we assumed.

To generate a binary predictor variable as to whether each species possess putative flashing signals, we first calculated the proportion of volunteers who agreed on their classifications. Based on the consistency of classification, we generated the species classification variable in several different ways. First, we used (i) the modal (majority) classificatory response of volunteers for all species, and then we increasingly restricted our analysis to (ii) only those species for which more than 70% of volunteers agreed on their classifications, (iii) only those species for which more than 80% agreed, (iv) only those species for which more than 90% agreed and (v) only those species on which all (100%) of our volunteers agreed on their classification. Each of these forms of classification retained 61 bird species (we removed the two species that the volunteers' responses were divided into half, to be conservative), of which 21 were classed as species with hidden signals, 55 species (of which 17 had hidden signals), 41 species (of which 13 had hidden signals), 24 species (of which 8 had hidden signals) and 10 species (5 with hidden signals). We performed PGLS on all variables that retained more than 20 species (i.e. all but 100% level of consistency). We appreciate that the uncertainty of classification may itself have biological meaning. To incorporate this uncertainty directly, we additionally fitted an analogous PGLS model, this time using the proportion of volunteers that classified a species as having hidden colour patches as a continuous predictor variable of the extent of flash.

To elucidate the strength of evidence for an association between body size and the presence of putative flashing signals as demonstrated in other taxa [29], we performed PGLS using log-transformed mean mass as a response variable and the presence of putative flashing signals as an explanatory variable (treated both in binary form and as a continuous predictor as above).

3. Results

(a) Human computer experiment

On fitting the generalized linear mixed model, there was evidence for an interaction between the prey item's FID that triggered movement and the prey's colour when mobile on whether it was attacked or not (full model with and without interaction, LRT $\chi_1^2 = 5.34$, $p = 0.028$; see electronic supplementary material, table S2 for separate main effect estimates and their interaction). When comparing the main effects model
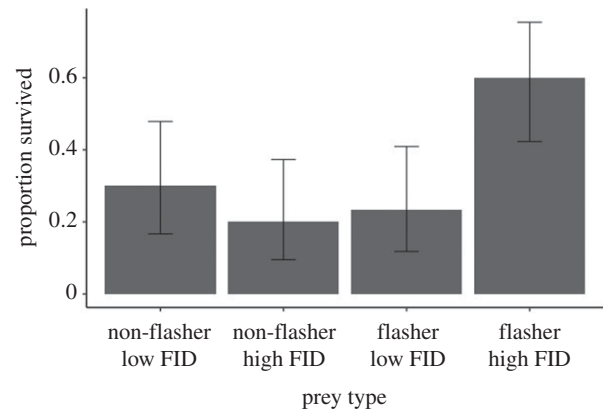

Figure 1. The mean proportion of each treatment prey type that survived once it had settled. The vertical error bars represent the 95% Wilson binomial confidence intervals for these means. FID: flight initiation distance.
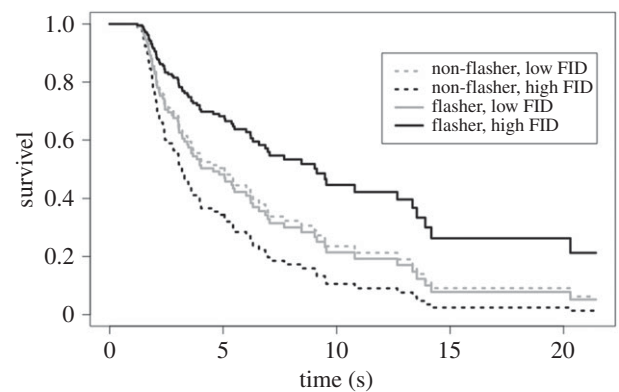

Figure 2. Distribution of the time taken by human volunteers to capture each of the four prey types. Those cases where the volunteer gave up searching were treated as censused at the time of giving up. Flashers with high FIDs tended to survive the longest, while non-flashers with high FIDs tended to survive the shortest period of time.

(i.e. the full model without interaction) with and without a given factor, then both the reactive distance (LRT $\chi_1^2 = 4.79$, $p = 0.029$) and whether it flashed (LRT $\chi_1^2 = 4.95$, $p = 0.026$) explained significant variation in whether the prey was ultimately attacked, as did the order of presentation (LRT $\chi_1^2 = 7.35$, $p = 0.007$) with the mean attack rate of all prey increasing over time. Collectively, flashers with long FID survived at about twice the rate as non-flashers in the experiment (figure 1; see electronic supplementary material, table S3 for full model estimates). Pairwise comparisons (see electronic supplementary material, table S4 for all comparisons) showed that flashing prey with high FID survived at a higher rate than all three alternative prey types (Tukey's pairwise multiple comparisons, all three p_{adj} less than 0.04), but no other pairwise comparison was significant (for the three remaining pairwise comparisons p_{adj} greater than 0.98).

Our survival analysis revealed borderline evidence of an interactive effect of FID and whether it flashed, on time to attack (full model with and without interaction, LRT $\chi_1^2 = 3.64$, $p = 0.056$). When comparing the main effects model with and without a given factor, then a model including reactive distance did not explain significant variability (LRT $\chi_1^2 = 0.81$, $p = 0.369$) but whether it flashed did explain significant variability in attack time (LRT $\chi_1^2 = 6.51$, $p = 0.01$) as did the order of presentation (LRT $\chi_1^2 = 8.00$, $p = 0.005$). Once again, prey that flashed only appeared to benefit from

Table 1. Results of PGLS models (assuming a BM model of trait evolution) predicting the FID of birds using the maximum clade credibility tree.

the way that the presence of flashing signal was inferred	predictor of FID	coefficient	t	p-value
the modal response of volunteers ($n = 61$ species)	flashing signal presence	0.11	3.16	0.004
	log (mass)	0.21	5.39	<0.001
	interaction	0.008	0.32	0.75
the proportion of volunteers that classified a species as having flashing signals ($n = 63$ species)	flashing signal presence	0.40	3.70	0.001
	log (mass)	0.19	4.51	<0.001
	interaction	−0.05	−0.86	0.42

flashing if their FID was long (figure 2). Thus, compared to flashing prey with a long FID, flashing prey with a short FID tended to be more vulnerable (hazard ratio 2.09, 95% CI 0.998–4.377). Non-flashing prey were even more susceptible to being attacked than the flashing prey with long FID (short FID, HR 2.539, 95% CI 1.173–5.497; long FID, HR 3.207, 95% CI 1.511–6.807).

(b) Phylogenetically controlled analysis

Here, we present the results that used (i) the modal response of volunteers as a binary flash predictor and (ii) the proportion of volunteers that classified a species as having putative flashing signals as a continuous flash predictor in the main results since they retained almost all species we studied. The results that used other criteria did not differ qualitatively from these results and can be found in electronic supplementary material, figure S3 and table S5. Although we iteratively performed PGLS analyses on 1000 trees, the 95% confidence limits of the estimated parameters and ranges of p-values from all trees were very narrow (±0.01 range in p-values and ±0.04 range in all coefficients and result statistics; electronic supplementary material, figure S4). Thus, we only present the results from the MCC tree which are essentially the same as the results from 1000 trees.

The FID of birds was significantly higher in both larger species and those species that have putative flashing signals (table 1, and figures 3 and 4). The interaction effect between body size and flashing signal presence on FID was non-significant. The results were consistent when we used the 70% agreed variable for the presence of hidden colour patches (electronic supplementary material, table S5). However, the effects of putative flashing signal presence became non-significant when we used both 80 and 90% agreed binary variables (electronic supplementary material, table S5 and figure S3), likely due to a reduction in statistical power or because the effect is driven by species with particularly ambiguous flash signals. The presence of putative flashing signals was also associated with larger size (coefficients = 0.25, $t = 2.18$, p-value = 0.05; see electronic supplementary material, table S6 for the additional results that used different explanatory variables for the presence of flashing signals).

4. Discussion

Our human experiment showed that the survival benefit of flash behaviour in artificial prey was dependent on the prey having a relatively long FID. Our complementary phylogenetic analysis was consistent with this inference, indicating
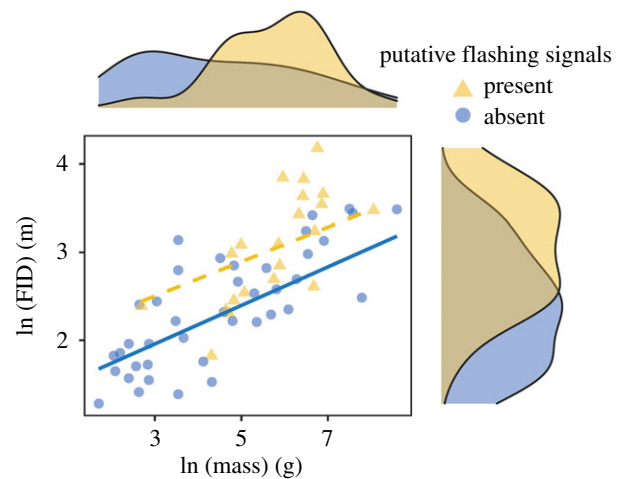

Figure 3. A scatterplot and density plots depicting the relationship between mean body mass, mean FID and the presence of putative flashing signals in birds. We used the modal response of volunteers when classifying each species as having putative flashing signals or not ($n = 61$ species) for plotting. The trend lines represent the predictions from the PGLS models using the MCC tree. (Online version in colour.)

that birds that have putative flashing signals tend to have relatively longer FID than those with no such signals, even after controlling for their body size. Collectively, our results demonstrate the importance of being undetected before fleeing in flashing prey and how this has shaped the evolutionary relationship between the employment of hidden signals and fleeing behaviour in birds.

Our finding that the anti-predation benefit of flash behaviour is dependent on FID provides additional mechanistic support for the hypothesis that flash behaviour serves as a decoy to deceive predators into assuming that the flasher is no longer present. In our human experiment, prey with longer FIDs were unlikely to have been seen prior to their movement. For non-flashing prey that had cryptic colours throughout, the FID appears to have been relatively inconsequential for its subsequent survivorship, because those prey were seen to be cryptic when in motion, so the searcher knew what to expect. By contrast, for those prey whose colour at rest differed from when in motion, not witnessing the prey item's original resting colour probably led participants to believe that the prey's 'flash colour' was also its colour at rest. When the prey returned to its resting state, participants not seeing the colour they had previously associated with the prey likely assumed it was not present and gave up searching.

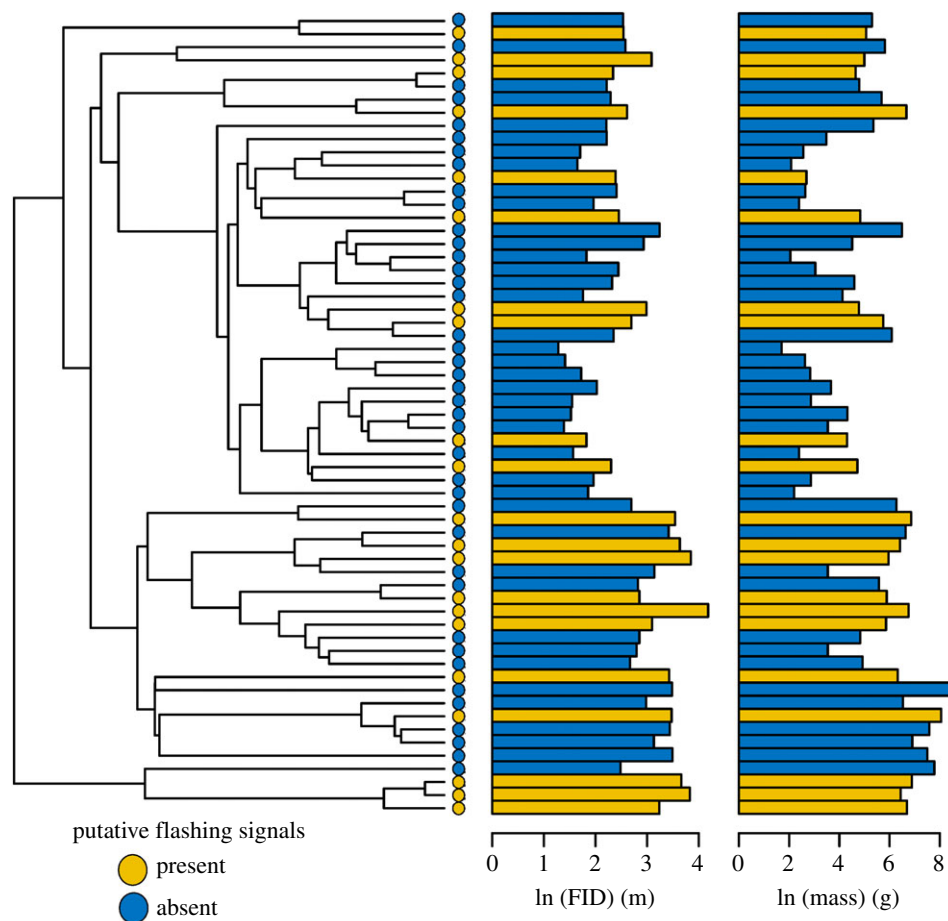Although flashing and non-flashing prey with short FIDs did not show significant survivorship differences in our

Figure 4. Mean body mass, mean FID (both logged) and the presence of putative flashing signals of each species and their phylogenetic positions on the maximum clade credibility tree. (Online version in colour.)

game, in nature there may be some costs associated with flash displays which may favour non-flashing in these cases. Such costs might include possible metabolic costs involved in the generation of high contrast colours [37] and/or the potential for increased conspicuousness to predators by an incidental revealing of flash colours while at rest. Thus, flash displays may be selected against in species where their decoy function is rendered ineffective by a low FID.

It has previously been speculated that flash behaviour may function to simultaneously mislead predators as to the prey's actual resting appearance and function as a startle signal [3,4]. Our findings call this supposition into question. Startle signals deter predation by inducing a reflexive fear response that causes the predator to hesitate or flee, buying time for the prey to escape [7]. For a startle signal to induce such a response, the predator must perceive an imminent threat. If the startle signal is relatively far away, the perception of threat may be significantly reduced because real threats inherently become less dangerous with increasing distance. Given our finding that putative flash signals tend to be deployed when the predator is far away, it appears unlikely that the putative flash colours also serve as a startle.

There are a variety of plausible alternative anti-predation mechanisms that may cause birds to evolve contrasting hidden signals [8,38,39]. Brooke [40] found that species of wader with flash marks are more likely to flock and proposed three inter-related predation-based explanations for the association, namely (i) it has evolved as a signal to conspecifics to flee, (ii) it generates a confusion effect in grouped prey and (iii) it serves to coordinate flight. Intriguingly, Mayer *et al.* [41]

found that groups of certain species of waterbird tend to have longer FIDs than solitary conspecifics and proposed that this relationship is caused by groups of birds being more able to detect an approaching threat. The FID data we analysed [24] were based on individual focal birds and we did not include group size or flocking tendency as a predictor in our phylogenetic model. Given the above, it is possible that flocking tendency may have coevolved with flash signals to increase FID, but whether the decoy effect works at the group level remains to be determined.

An additional plausible explanation for hidden signals is that the flashing colours of some birds function to reduce the likelihood of the signaller being captured during its pursuit [8,39]. In these cases, whether a bird is seen to have a different appearance at rest and in flight would be unimportant. While the association we observed between FID and flash displays can be readily directly explained on the basis of deception (see above), it remains possible that bird species that experience higher predation pressure face elevated selection for both hidden contrasting signals and relatively long FID independently. In some cases, the hidden contrasting colours that we considered putative flash signals may, in fact, operate as pursuit deterrent signals. Indeed, there is evidence that pursuit deterrent signals have coevolved with FID in some species of lizard [42–44]. Intriguingly, our phylogenetic analysis also revealed that larger species of bird are more likely to have hidden signals, just as larger species of insect are also more likely to have hidden signals [5,10,45]. This probably reflects higher selective pressure to evolve a secondary defence on large species because of higher conspicuousness and/or higher caloric profitability. However, there is evidence that

flash behaviour may be more effective at deterring predation in large prey because smaller species are already hard to detect, and thus selection to evolve a back-up defence is weaker in these species [11].

Other explanations for flash displays can be ruled out. In particular, while males and females of a given bird species sometimes differed in appearance in our data, they were consistently classified in the same way with respect to flash coloration. This indicates that sexual selection is not the basis of selection for hidden colour signals in birds. Indeed, it has similarly been noted that not a single instance of sexual variation in the presence of hidden signals was reported out of a sample of over 600 species of insects, spanning six clades [9,10]. Likewise, thermal regulatory factors also influence the evolution of animal coloration [46]. However, patches of colour only visible while in flight likely have a negligible effect on thermoregulation. Therefore, we consider it unlikely that selection for thermally favourable coloration has confounded our results.

To our knowledge, this is the first study to compare the FIDs of species that display contrasting signals when fleeing (i.e. flashing) and those that do not. However, one potential limitation of the data used in our phylogenetic analysis is that the categorization of birds as having or not having hidden contrasting colours was carried out in the human visual range, and not the visual range of ecologically relevant predators. The birds in this study are all native to Australia, which is largely devoid of native mammalian predators. Therefore, the dominant selective pressure likely to have shaped the evolution of anti-predation traits in our study species is predation by other birds, which may be better able than mammals to pursue escaping avian prey. Birds are known to perceive all colours within the human visual range as well as colours in the UV region (300–400 nm wavelength region that humans cannot perceive) [47], but recent studies suggest that birds and humans do not differ substantially in discriminating colour differences [48,49]. Therefore, all of the birds that we classified as contrasting (in the visible range) would have contrasted to their avian predators as well. Some of the birds that we classified as non-contrasting may display some degree of contrast to avian predators, especially when UV is associated. Given that flash coloration may function by drawing attention to the signaller, it seems unlikely that selection would favour UV-only signals for flash behaviour.

FIDs are important metrics of perceived risk and have long been documented by conservation biologists and ecologists. Our classification of birds was based on whether they reveal hidden colours when fleeing and we did not attempt to quantify their extent of crypsis at rest, which is background specific and varies with light conditions. It is currently unclear whether more conspicuous birds tend to compensate for their higher conspicuousness by fleeing at a greater distance. One study to address this question found no evidence that the FIDs of birds was associated with their vividness of colour at rest [50]. However, Møller et al. [51] recently found that the mean FIDs of 12 bird species assessed to be 'with camouflage' were significantly shorter than their matched 12 sister taxa without camouflage. Our finding of an association between FID and exposure of hidden signals while fleeing would not be evident in the above analyses that are based simply on the extent of crypsis in resting plumage alone.

Our study highlights a coevolutionary relationship between plumage coloration and FID in birds and provides evidence for a plausible underlying mechanism. Many other taxonomic groups exhibit flash behaviour, including insects and mammals, and given the simplicity of the explanation it would be of interest to determine whether they show the same relationship. For example, Butler recorded the FIDs of nine species of grasshopper [13]. While there are too few species for meaningful statistical comparisons, it is notable that all of the species with hidden contrasting colours (only visible in flight) had longer median FIDs than those without (electronic supplementary material, figure S5). Artiodactyla is another promising group to evaluate since many species display colours that are only visible when they flee, although the range of different ways of measuring FID makes cross-species comparisons challenging at this time [17].

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