

Quantifying the effects of species traits on predation risk in nature: A comparative study of butterfly wing damage

Freerk Molleman^{1,2,3}  | Juhan Javoš³  | Robert B. Davis³  | Melissa R. L. Whitaker^{4,5} |
 Toomas Tammaru³  | Andreas Prinzing⁶  | Erki Õunap³ | Niklas Wahlberg⁷  |
 Ullasa Kodandaramaiah² | Kwaku Aduse-Poku^{8,9} | Ants Kaasik³ | James R. Carey¹⁰

¹Department of Systematic Zoology, Faculty of Biology, Institute of Environmental Biology, A. Mickiewicz University, Poznań, Poland; ²IISER-TVM Centre for Research and Education in Ecology and Evolution (ICREEE), IISER Thiruvananthapuram, Thiruvananthapuram, India; ³Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia; ⁴Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; ⁵Entomological Collection, ETH Zürich, Zürich, Switzerland; ⁶Université de Rennes 1, Ecosystèmes Biodiversité Evolution (ECOBIO), Rennes, France; ⁷Department of Biology, Lund University, Lund, Sweden; ⁸Department of Biology, University of Richmond, Richmond, VA, USA; ⁹Department of Zoology, University of Cambridge, Cambridge, UK and ¹⁰Department of Entomology, University of California, Davis, Davis, CA, USA

Correspondence

Freerk Molleman

Email: fremol@amu.edu.pl

Funding information

Centre National de la Recherche Scientifique, Grant/Award Number: ATIP; Bixby International Travel Grant; Région Bretagne; European Regional Development Fund; Estonian Science Foundation, Grant/Award Number: 9215 and IUT20-33; National Institute on Aging, Grant/Award Number: PO1 AG022500-01 and PO1 AG608761-10; Department of Science and Technology, Government of India, Grant/Award Number: DST/I and NSPIRE/04/2013/000476

Handling Editor: Jean-Michel Gaillard

Abstract

1. Evading predators is a fundamental aspect of the ecology and evolution of all prey animals. In studying the influence of prey traits on predation risk, previous researchers have shown that crypsis reduces attack rates on resting prey, predation risk increases with increased prey activity, and rapid locomotion reduces attack rates and increases chances of surviving predator attacks. However, evidence for these conclusions is nearly always based on observations of selected species under artificial conditions. In nature, it remains unclear how defensive traits such as crypsis, activity levels and speed influence realized predation risk across species in a community. Whereas direct observations of predator–prey interactions in nature are rare, insight can be gained by quantifying bodily damage caused by failed predator attacks.
2. We quantified how butterfly species traits affect predation risk in nature by determining how defensive traits correlate with wing damage caused by failed predation attempts, thereby providing the first robust multi-species comparative analysis of predator-induced bodily damage in wild animals.
3. For 34 species of fruit-feeding butterflies in an African forest, we recorded wing damage and quantified crypsis, activity levels and flight speed. We then tested for correlations between damage parameters and species traits using comparative methods that account for measurement error.
4. We detected considerable differences in the extent, location and symmetry of wing surface loss among species, with smaller differences between sexes. We found that males (but not females) of species that flew faster had substantially less wing surface loss. However, we found no correlation between cryptic coloration and symmetrical wing surface loss across species. In species in which males appeared to be more active than females, males had a lower proportion of symmetrical wing surface loss than females.

5. Our results provide evidence that activity greatly influences the probability of attacks and that flying rapidly is effective for escaping pursuing predators in the wild, but we did not find evidence that cryptic species are less likely to be attacked while at rest.

KEYWORDS

activity, ageing, crypsis, defensive ecology, flight speed, restricted maximum likelihood, sex differences, symmetrical damage

1 | INTRODUCTION

Predation risk is inevitably dependent on prey defensive traits such as behaviour and appearance (Ruxton, Sherratt, & Speed, 2004). For instance, studies show that crypsis reduces attack rates on resting prey, that prey activity increases predation risk and that rapid prey movement increases chances of surviving predator attacks (e.g. Husak, 2006; Ioannou & Krause, 2009). However, evidence for these conclusions usually comes from observations of selected species under artificial conditions (e.g. Prudic, Stoehr, Wasik, & Monteiro, 2015) or artificial prey in the wild (e.g. Stevens, Hardman, & Stubbins, 2008). The extent to which species traits influence realized predation risk across species in a natural community remains largely unresolved (Rommel, Davison, & Tammaru, 2011).

One reason for the limited empirical evidence relating prey traits with predation rates is the transient nature of predator–prey interactions, such that predator attacks on wild prey are not observed frequently enough to be reliably quantified. However, since most predator attacks are unsuccessful and these failed attacks often cause permanent and observable damage, the study of patterns of bodily damage in wild animal populations offers an alternative approach for understanding the links between prey traits and predation risk (Edmunds, 1974; Raffaelli, 1978; Rand, 1954; Robbins, 1981; Schoener, 1979; Vermeij, 1982).

The locomotory behaviour of prey interacts with the effectiveness of various other types of defences. For example, crypsis is an effective anti-predator trait when individuals are at rest, but not when moving (De Ruiter, 1952; Ioannou & Krause, 2009; Stevens, Searle, Seymour, Marshall, & Ruxton, 2011). Furthermore, the proportion of time that prey spends moving is an important determinant of predator attack rate (Bieber & Ruf, 2009; Skelly, 1994), because most predators predominantly target moving prey (Hailman, 1977). During movement, the outcome of attacks likely depends largely on the prey's escape ability, which is often a function of its speed (e.g. Heithaus, Frid, & Dill, 2002). Since particular body parts are differentially exposed while the prey is at rest as opposed to when in motion, the type and configuration of bodily damage likely differ in individuals that were attacked while resting compared with those that were attacked during activity. The location and configuration of damage can therefore be indicative of the behavioural context of attacks (Arlet, Carey, & Molleman, 2009).

Butterflies are particularly suitable for studies linking damage patterns, species traits, and predator attack rates and outcomes. Damage to butterfly wings in the shape of a bird's beak has been interpreted as an outcome of failed predation attempts by birds (Wourms & Wasserman, 1985). Therefore, relative frequencies of beak marks across butterfly species contain information about the effects of butterfly species traits on predation by birds. For example, beak mark frequencies have been shown to be correlated with butterfly palatability (Carpenter, 1941; Ohsaki, 1995). The location of wing damage can also be correlated with butterfly defence traits. Robbins (1981) and Tonner, Novotny, Lepš, and Komarek (1993) showed that butterfly species with false heads (hindwing coloration and shape that give the impression that the head is on the posterior end of the animal) had damage more often on hindwings than on forewings, compared to species without such markings. Furthermore, it can be viewed that symmetrical wing surface loss is incurred while butterflies are at rest, because most butterfly species keep their wings closed while at rest, while they are necessarily open during flight (Shapiro, 1974). Thus, the frequency, location and symmetry of butterfly wing damage all contain information about predator–prey interactions.

In addition to differences among species, there are intraspecific differences between the sexes in activity, body size and allometry, coloration, wing shape and anti-predator tactics in many butterfly species (e.g. Fermon, Waltert, & Mühlenberg, 2003; Ide, 2006; Ohsaki, 1995; Owen & Smith, 1990; Wallace, 1889; Westerman et al., 2019). For example, flight morphology can be in part shaped by mate locating behaviour and may therefore differ between the sexes (Wickman, 1992). We expect that species with larger differences between the sexes in defensive traits should also show larger differences between the sexes in damage patterns.

Of course, animals are also subject to general wear and tear, which generates damage that is not attributable to predator attacks. In butterflies, wing scales are gradually lost with age, whereas wing surface loss is more often due to attacks by predators (Kemp, 2001; Robbins, 1981). Therefore, we expect stronger within-individual correlations between damage that is likely caused by predator attacks across different wings (e.g. wing surface loss inflicted during a single attack), than between predator-related damage (e.g. wing surface loss) and damage that is likely due to wear and tear (e.g. scale loss).

Despite the intuitiveness of the effects of butterfly traits on damage patterns, quantitative aspects of such relationships remain

largely uncharacterized. In particular, crypsis and flight speed are thought to be important defences for the majority of butterfly species, but quantitative data regarding their effectiveness in the wild are sorely lacking. Previous wing damage studies have focused on particular defence traits that were not quantified on a continuous scale, considered few species or did not account for phylogeny.

In the present study, we quantify the effectiveness of defence traits by testing for correlations between damage parameters and species traits (flight speed and activity, importance of crypsis as a defence tactic), using data taken from 34 species of fruit-feeding butterflies in a tropical forest in Uganda. These species differ in body size, activity levels and anti-predator traits. We assume that they largely share the same community of potential predators because they occur in the same habitat, even though they may, for example, use different forest strata (Molleman, Kop, Brakefield, DeVries, & Zwaan, 2006). We examined the overall distribution of wing damage in fore- and hindwings and tested for correlations between damage parameters within individual butterflies. We then evaluated the potential for species traits to affect damage patterns by assessing among-species variation in the presence, extent, location and symmetry of wing surface loss, the number of tears in wings and the extent of wing scale loss. We explored correlations across species using a restricted maximum likelihood (REML) approach that accounts for both phylogenetic non-independence of species and measurement error (Ives, Midford, & Garland, 2007) when possible. We included wing length (a proxy of body size and the wing area exposed to predators), as a predictor of wing damage parameters. We also used the residuals of wing damage over wing length in an alternative analysis, because other species traits may be confounded with body size (e.g. larger butterflies tend to fly faster). We tested the following hypotheses: (a) faster flying species have less wing surface loss overall (because faster species are more likely to escape pursuing predators), (b) species that rely more on crypsis for defence have a lower frequency of symmetrical damage compared with those that rely more on other anti-predator traits (because they are less frequently spotted while resting with wings closed), and (c) in species in which males are more active than females, males have less symmetrical surface loss than conspecific females (because males are more often attacked while their wings are open).

2 | MATERIALS AND METHODS

2.1 | Study system and timing

This study was conducted near the Makerere University Biological Field Station in Kibale National Park, Western Uganda. Butterflies were captured in fruit-baited traps in two areas with selectively logged sub-montane tropical forest (Lowercamp and K31) and a forest regeneration site (Mikana). We used 22 trap locations in Lowercamp (Molleman et al., 2006), 40 trap locations in the understorey of forest compartment K31 and six trap locations in the Mikana area. In K31, traps were baited once a week from January

2006 until February 2007, and butterflies were scored, marked and released on four consecutive days between 10:00 and 16:00, replacing bait only when it was lost. In Lowercamp and Mikana, trapping was performed once every 4 weeks from May 2006 to June 2012. Since the traps accumulated butterflies over 24-hr time periods, any differences in diurnal activity could not bias trap catches.

In forest compartment K31, 34 species of fruit-feeding butterflies were included to capture as much diversity in terms of phylogeny and putative defensive tactics, as possible. In Lowercamp and Mikana, we focused on three butterfly species: *Euphaedra medon* (L.), *E. alacris* Hecq and *Charaxes fulvescens* Aurivillius in order to obtain large sample sizes for selected species. We focused on medium-to large-bodied species that are less likely damaged by handling. All included species hold their wings closed over their back while at rest and are thus expected to show symmetrical wing damage if they were attacked while at rest, although the *Adoliadini* and *Cymothoe* species hold their wings open during sun basking and can open their wings during feeding (FM pers. obs.).

2.2 | Scoring damage

Focal species were carefully removed from baited traps by hand. To avoid pseudo-replication, butterflies were marked with a unique number before release. Most individuals were captured only once (the proportion of captures that were recaptured is given in Table B3 and the frequency of recaptures in Table B4 of Appendix S2). We visually estimated the proportion of wing surface missing on each wing as well as the percentage of scale loss of all wings taken together. Any entire number could be noted, albeit obviously a difference of 1% would not be interpretable. We compared estimates of wing surface loss with detailed drawings of the wing surfaces of 538 of the included specimens and corrected systematic biases accordingly (e.g. overestimation of minor damage, underestimation of severe damage: Appendix S2). We also counted the number of tears (ripped wings without surface loss) in the wings (Figure 1). To gauge the realized repeatability of estimates of butterfly wing damage in this study, we took data from individual butterflies that were captured and recaptured at most 1 day apart (estimates often made by different observers) and determined the correlation between the two estimates of wing damage. Since the butterflies could have incurred new wing damage during this 1 day, it is likely that we slightly underestimate repeatability. Across 1,100 instances of individuals that were captured on two consecutive days, the correlation coefficient of wing surface loss was 0.74 on average, wing tears 0.53 and scale loss 0.98. We note that stronger correlation, that is reproducibility, did not correspond to stronger statistical signal in the later tests of our hypotheses (Table 1). We calculated the degree to which wing surface loss was biased towards forewings as the damage to forewings minus that in hindwings, divided by the total wing surface loss; such that this variable had positive values when wing surface loss was biased towards forewings and negative values when biased towards



FIGURE 1 Representative examples of wing damage types; (a) non-symmetrical wing surface loss (~5% of left hindwing) in the shape of bird's beak (*Gnophodes chelys* female), (b) irregularly shaped non-symmetrical wing surface loss (~20% of right hindwing and 3% of right forewing *Kallimoides rumia* female), (c) symmetrical and non-symmetrical wing surface loss in hindwings (~10% of left hindwing, 3% of right hindwing *Euphaedra eusemoides*), (d) symmetrical fore- and hindwing surface loss and non-symmetrical hindwing surface loss (*E. alacris* female), (e) tears in fore- and hindwings (*E. alacris* female) and (f) severe scale loss (~50%) and slight (~5%) surface loss to all wings (*E. edwardsi*). We did not handle butterflies as in plate c during this study

hindwings. For each pair of wings, we scored whether any of the surface missing was symmetrical (i.e. the surface loss on left and right wings represented a mirror image of each other). Even when some of the wing surface loss had a symmetrical shape across wing pairs, the extent of wing surface loss of wings in a wing pair often differed between the two wings, because there was additional non-symmetrical wing surface loss.

We attempted to avoid damage due to handling by focusing on species of large body size (forewing length over 2.8 cm.) and by working with local field assistants with several years of experience in handling butterflies. Fingerprints on butterfly wings are readily recognizable and were ignored when scoring butterflies. We noted if a specimen was damaged during handling and excluded any subsequent recaptures of these individuals from the analyses.

2.3 | Quantifying species traits

For each species and sex, we determined averages and standard errors (SE) of trait values. These were then used as predictors of sex-specific species averages of wing damage parameters. Forewing measurements were made using callipers at the study site for the 34 species, represented by 12,271 live individuals that were not included in the study of damage (separate dataset). Sexual size dimorphism was calculated as the log of the ratio of male to female wing length ($\log(\text{wing length male}/\text{wing length female})$). Based on Taylor expansion, we approximated the standard error (SE) of sexual size dimorphism as $\sqrt{(\log(1 + (\text{measurement error of males}/\text{mean for males})^2) + \log(1 + (\text{measurement error of females}/\text{mean for females})^2))}$.

Three experts in butterfly natural history (Perpetra Akite, Torben B. Larsen and Freerk Molleman) ranked the species they were familiar with according to the importance of crypsis as an anti-predator trait relative to other defences on a scale of zero to one, and these

ranks were then averaged (Appendix S2, Table B1). These opinions took into account both ventral and dorsal surfaces. We acknowledge that these estimates were based on human perception, which might differ from that of actual predators of butterflies (e.g. Church et al., 1998; Walton & Stevens, 2018), and averaging from incomplete rankings may cause slight distortions.

For most species, flight speed was measured in a 3-m-long tunnel. A house at the field station was darkened except for one exterior door that was left open, and the doorframe was covered with white mesh, providing a light target to butterflies. Butterflies were taken from baited traps in the morning during a 4-month period, provided water and mashed banana and used during the afternoon between 13:00 and 16:00 of the same day for flight speed measurements. Therefore, the ambient temperature was roughly the same for all trials, ranging between 20.5 and 25°C. Butterflies were individually released 1 m from the floor and 4 m from the open door, oriented towards the open door. Butterfly flight away from a human experimenter is likely escape behaviour; thus, we presume that butterflies were displaying escape flight tactics and were ostensibly maximizing their speed. The time they took to reach the mesh covering the open door was recorded, and flights that were not straight towards the target door were excluded from analyses.

As a measure of sexual differences in foraging activity, we calculated the proportion of males and females captured in baited traps during 12.5 years of butterfly monitoring (trap ratio) using a separate dataset: Molleman et al. (2006), Valtonen et al. (2013) and unpublished data. This included the study areas described above as well as four other nearby sites varying from nearly pristine forest to regenerating sites, and including canopy traps that were monitored from 2000 to 2006 (partly in Molleman et al., 2006). The trap ratio was not calculated for species that are difficult to sex in the field. Notably, the sexes may differ in their responses to trap height and their tendency

TABLE 1 Correlation coefficients for wing damage variables putatively inflicted by predators and species traits across species of fruit-feeding butterflies in a forest in Uganda

	Surface loss	SuLo Loc	Tears	Tears Loc	Scale loss	Prop FW SuLo	Prop HW SuLo	Pr sym FW SuLo	Pr sym HW SuLo
Males									
Wing length	0.44 (-0.01, 0.82)	*0.61 (0.26, 0.84)	*0.72 (0.43, 0.93)	*-0.83 (-1.00, -0.49)	-0.14 (-0.48, 0.26)	0.17 (-0.21, 0.51)	0.21 (-0.14, 0.52)	-0.04 (-0.40, 0.35)	-0.13 (-0.47, 0.23)
Speed	-0.71 (-0.96, -0.30)	-0.16 (-0.68, 0.46)	-0.51 (-0.86, 0.07)	0.15 (-0.85, 1.00)	0.07 (-0.43, 0.53)	0.12 (-0.37, 0.56)	-0.44 (-0.77, 0.02)	0.23 (-0.26, 0.66)	-0.41 (-0.74, 0.05)
Crypsis	0.18 (0.41)	-0.14 (0.37)	0.28 (0.11)	-0.53 (0.001)	-0.06 (0.79)	0.01 (0.97)	0.41 (0.04)	-0.32 (0.13)	0.17 (0.48)
Females									
Wing length	0.43 (-0.16, 0.85)	0.53 (-0.11, 0.93)	0.40 (-0.07, 0.80)	-0.79 (-1.00, -0.31)	0.20 (-0.20, 0.54)	0.19 (-0.49, 0.80)	0.32 (-0.31, 0.85)	0.41 (-0.02, 0.76)	-0.65 (-1.00, -0.14)
Speed	0.16 (-0.54, 0.81)	-0.24 (-0.92, 0.63)	0.26 (-0.42, 0.79)	-0.28 (-1.00, 1.00)	0.42 (-0.14, 0.79)	-0.17 (-0.92, 0.76)	0.36 (-0.48, 0.95)	-0.25 (-0.80, 0.43)	-0.39 (-0.96, 0.54)
Crypsis	0.27 (0.16)	-0.06 (0.60)	0.36 (0.04)	-0.17 (0.31)	-0.20 (0.45)	0.40 (0.04)	0.20 (0.25)	0.22 (0.20)	-0.09 (0.97)
Sex diff.									
Trap ratio	1.00 (-0.66, 1.00)	1.00 (-1.00, 1.00)	*1.00 (0.71, 1.00)	-1.00 (-1.00, 1.00)	0.78 (-0.15, 1.00)	0.08 (-0.41, 0.55)	0.56 (-0.12, 0.99)	*0.75 (0.41, 0.94)	0.54 (0.06, 0.85)
WL SSD	-1.00 (-1.00, 1.00)	-1.00 (-1.00, 1.00)	-1.00 (-1.00, 1.00)	1.00 (-1.00, 1.00)	1.00 (-1.00, 1.00)	-0.08 (-0.57, 0.45)	-0.41 (-0.94, 0.36)	0.62 (-0.17, 0.90)	0.44 (-0.14, 0.81)

Note: For wing length and flight speed, we use a restricted maximum likelihood approach, taking into account phylogenetic non-independence and measurement error and report 95% CI. For crypsis, *p*-values from phylogenetic independent contrasts are provided. Correlations that are significant at the *p* < .05 level are in bold, with an * when the relationship was still significant after Bonferroni-Holm correction. Su Lo = percentage wing surface loss, Loc = the bias of wing damage to hind wings, Tears = the number of tears in wings, FW = forewing, HW = hindwing, Pr = the proportion of individuals with wing surface loss, Pr, sym = the proportion of individuals that have any wing surface loss that is symmetrical (from those individuals that do show wing surface loss in the particular wing pair), trap ratio is the ratio of males and females in traps. WL SSD = sexual size dimorphism of wing length. Results using residuals of wing damage over wing length are given in Appendix S2, Table B3.

to remain trapped after entering a trap (Alexander & DeVries, 2012); therefore, we excluded species known to show marked sexual differences in flight altitude from the analyses, reducing the sample size for this analysis to 31 species. It is also possible that within butterfly species, males and females differ in their seasonal foraging activity, as previously shown in a Mexican dry forest (Torres, Osorio-Beristain, Mariano, & Legal, 2009). Nevertheless, this effect is likely offset by the long duration of our study (1–6 years) and may not be as pronounced given the moderate seasonality of the butterfly community at our study site (Valtonen et al., 2013). We further assume that sex ratios in traps represent differences in foraging activity rather than differences in actual abundance such as might be caused by differential larval or adult survival. Indeed, a mark–recapture study showed sexual differences in foraging activity of African fruit-feeding butterflies (Fermon et al., 2003), and we have observed similar differences in the relative recapture rates of males and females of some species in the present study community (Molleman, Zwaan, Brakefield, & Carey, 2007, Appendix S2) which are most plausibly due to differences in foraging activity. The SE of the sex ratio was calculated as $((\text{ratio} \times (1 - \text{ratio})) / \sqrt{\text{N (individuals)}})$, unless a proportion was 0 or 1, in which case we calculated SE as $0.25 / \sqrt{\text{N individuals}}$, the highest possible SE.

2.4 | Reconstructing the phylogeny of the study community

To construct a phylogenetic tree and estimate divergence times, we used published (Aduse-Poku, Brakefield, Wahlberg, & Brattström, 2017; Aduse-Poku et al., 2015; Aduse-Poku, Vingerhoedt, & Wahlberg, 2009; Kodandaramaiah et al., 2010; Kodandaramaiah & Wahlberg, 2007; Monteiro & Pierce, 2001; Van Velzen, Bakker, & Loon, 2007; Van Velzen, Wahlberg, Sosef, & Bakker, 2013) and original DNA sequences for nearly all fruit-feeding butterfly species in the study community (96 species) with the help of calibration points (see Appendix S1 for more details). Including more species at the tree-building stage enabled us to produce a better-resolved phylogeny in the first instance, but the resulting tree was pruned to represent only the 34 butterfly species in this study (Figure 1 in Appendix S1).

2.5 | Statistical analysis

We first explored how different types of wing damage co-occur within individuals across all species (e.g. to what extent is wing surface loss in one wing of an individual correlated with wing surface loss in the other?) by implementing bivariate linear mixed-effect models using the 'rmcorr' function in R (Bakdash & Marusich, 2017). Since differences in averages among species and the sexes can obfuscate within-individual correlations across all species (Bakdash & Marusich, 2017), we used species*sex as 'individuals' in the analyses using data from 11,774 individual butterflies. Since some individuals were captured multiple times, we used only one randomly selected observation per individual to ensure that all individuals were sampled equally.

We then calculated means and SE of damage parameters per species and sex. For continuous variables, we used mixed models with individual identity as a random effect and species–sex combinations as factors. By using individual identity as a random effect, we were able to retain information contained in multiple captures, but individuals that were captured more often could not bias the species average. Similar models for binary data (presence/absence of wing surface loss, presence/absence of symmetrical wing surface loss) did not converge. Therefore, we calculated for each individual for each of these parameters the proportions of observations of 'presence'. We then averaged these continuous proportions within species*sex. The SE of the resulting proportions was then calculated as $((\text{proportion} \times (1 - \text{proportion})) / \sqrt{\text{N individuals}})$. When a proportion was 0 or 1, we calculated SE as $0.25 / \sqrt{\text{N individuals}}$, the highest possible SE.

We conducted phylogenetic comparative analyses to study correlations between wing damage parameters and species traits, as well as between intraspecific sex differences in wing damage parameters, foraging activity and body size. When SE was available for both damage parameters and species traits, we considered SE using a REML framework based on theory developed by Ives et al. (2007) implemented in R (Davis, Javoš, Kaasik, Önap, & Tammaru, 2016; Holm et al., 2016; R Core Team, 2014). This approach effectively gave more weight to species for which we had a higher sample size. This was particularly important in our study because the extent of damage varied greatly among individuals, and sample sizes varied greatly among species. Since no measurement error could be calculated for crypsis, we used phylogenetic independent contrasts (Felsenstein, 1985) implemented in the R package ape (Paradis & Strimmer, 2004) when addressing the effects of this variable. To account for any effects of body size on damage parameters, we calculated the residuals (and their measurement errors) of damage over wing length in a phylogenetic context for each species and sex, and also correlated these residuals with species traits. To account for multiple testing, we applied the Bonferroni–Holm correction to each butterfly trait with p -critical .05.

3 | RESULTS

3.1 | Overall damage distribution and within-individual correlations

We scored damage for 16,901 individual butterflies belonging to 34 species, with 23 species represented by more than 100 records (Dryad data Molleman et al., 2019). Many individuals had no damage, and few individuals had severe damage (Figure B1 in Appendix S2). In 82% of individuals, some wing surface was recorded as missing, and in 35%, at least one wing was recorded as torn. As extremes, one butterfly had as much as 80% of the surface of a single wing missing, and another was missing 40% of its total wing surface. Averaged over the species averages, 51% of females had forewing surface loss and 80% hindwing surface loss, while among males, 46% had forewing surface loss and 74% hindwing surface

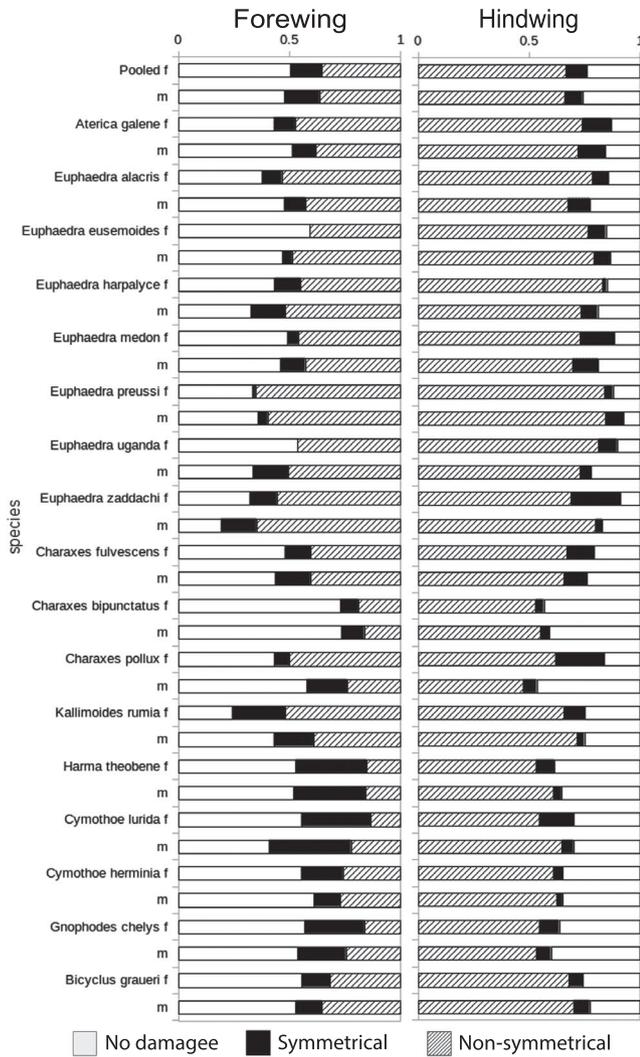


FIGURE 2 Proportion of individuals with and without (symmetrical/non-symmetrical) wing surface loss, presenting only species for which at least 30 females were scored. To illustrate that more closely related species tend to have more similar damage parameters, species are sorted by phylogenetic affiliation. All species had more frequent surface loss in hindwings than in forewings, and symmetrical damage was usually more common in forewings. These proportions varied more among species than within species among the sexes

loss (Figure 2). Given the high number of individuals ($df = 11,772$), within-individual correlations between different types of damage or between damage to different wings tended to be significant. However, the correlation coefficients (r) varied widely among combinations of damage parameters. Scale loss was weakly correlated with wing surface loss ($r = .20, p < .001$; Appendix S2, Figure B1) and with the number of wing tears ($r = .12, p < .001$). The average correlation between the extent of wing surface loss and the number of wing tears within individuals was also weak ($r = .11, p < .001$). In contrast, the percentage of surface loss was correlated more strongly between the two forewings ($r = .41, p < .001$). However, this was not the case for hindwings ($r = .02, p = .013$). This implies that if the left forewing of an individual was missing some surface,

the right forewing was likely to miss some surface too, but that this was not the case for hindwings. This is consistent with symmetrical wing surface loss being more common in forewings than in hindwings: when wings are held together, both wings are likely damaged in an attack. A similar trend was found for wing tears ($r = .20, p < .001$ for forewings and $r = .01, p = .160$ for hindwings).

This bias was especially strong in the Limentidini, Mycalesini, Melanitini and the one Kallimini, but absent or even slightly reversed in Adoliadini (see Figure 2 and Table B3 for species-specific information).

3.2 | Correlations between damage parameters and species traits

Differences among species in damage parameters were extensive (Appendix S2, Figure 2, Table B3). Considering phylogeny and, when possible, measurement error, we found correlations among species traits and damage parameters (Table 1 and Table B2 in Appendix S2). Most correlations were found in males, for which sample sizes tended to be larger, while females generally had similar correlation coefficients than males, with the notable exception of the correlation between wing surface loss and flight speed (Table 1).

There were three correlations between damage parameters and wing length (Table 1). Individuals of butterfly species with longer wings had wing surface loss that was more biased towards forewings (REML significant in males with $r = .61$; Table 1). Longer wings were also associated with more wing tears (significant for males with $r = .72$) that were more biased towards hindwings (significant for males with $r = -.83$ and females with $r = -.79$).

Among males, faster flying species had less wing surface loss ($r = -.71$), but no such trend appeared among females (Table 1, Figure 3). This relationship was significant when tested individually (an a priori prediction), but not when the Bonferroni-Holm correction was applied to account for multiple testing. Faster flying species also had less wing surface loss after wing length was taken into account (Appendix S2, Table B2). The effect of flight speed appears to be substantial: the males of species that fly twice as fast have about 30% less wing surface loss on average (Figure 3, see confidence interval of slope in Table 1). This effect appears to be mainly due to a lower proportion of individuals with hindwing surface loss in faster flying butterflies (Table 1). Body size and wing shape are expected to affect flight speed (e.g. Srygley, 1990). In our data, wing length was not significantly correlated with flight speed for males (REML: $r = -.15, 95\% \text{ CI } -0.59, 0.33$), but larger females were shown to fly faster ($r = .73, 95\% \text{ CI } 0.37, 0.92$). Wing shape (length/width) was not correlated with flight speed in either sex (males $r = .23, 95\% \text{ CI } -0.29, 0.67$; females $r = .06, 95\% \text{ CI } -0.65, 0.69$), taking into account the effect of body size by considering residuals of flight speed over wing length. When wing length was accounted for, higher flight speed was also associated with significantly fewer tears in wings of males ($r = -.72$, Appendix S2, Table B2). Less wing surface loss in the hindwings of faster flying species supports our hypothesis

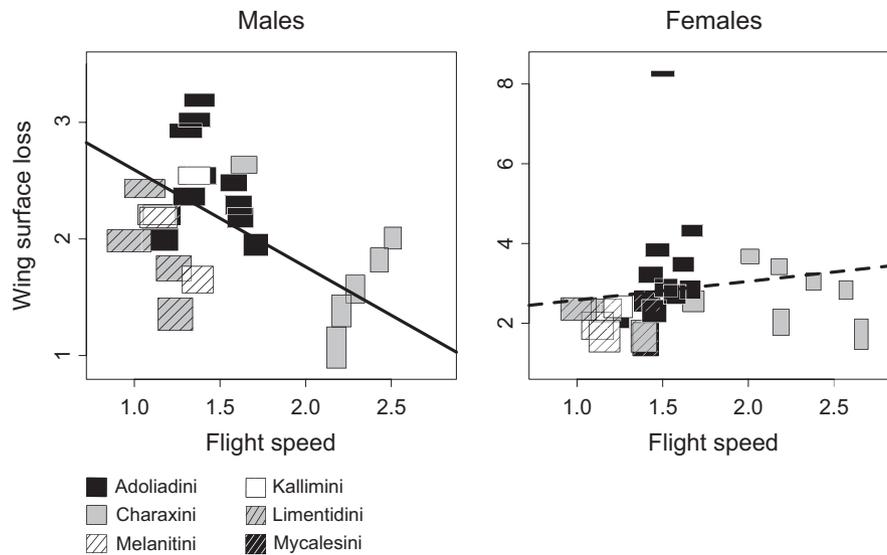


FIGURE 3 Raw correlations between flight speed and wing damage parameters that are putatively caused by predators across 34 butterfly species in a forest in Uganda. Solid lines indicate significant relationships; dashed lines are non-significant. Clades are pattern-coded, and relative sample size for each species trait is represented by the respective width and height of markers (within a graph). Trend lines are derived from analyses using a restricted maximum likelihood (REML) framework that takes into account phylogenetic non-independence and measurement error (statistical results in Table 1)

that faster flying butterflies more often escape pursuing predators without wing surface loss or are less frequently attacked in flight.

Species that were thought to rely more on crypsis as an anti-predator trait had neither more nor less wing surface loss than those that were thought to rely more on other defences (Figure 4; males $p = .41$, females $p = .16$). In contrast to our hypothesis that individuals that rely more on crypsis would be attacked less frequently while at rest and thus have a lower frequency of symmetrical wing surface loss, more cryptic species did not show a lower frequency of symmetrical wing surface loss (PIC: males, forewings $p = .13$, hindwings $p = .48$; females, forewings $p = .20$, hindwings $p = .97$; Table 1). Curiously, in species that rely more on crypsis, a higher proportion of individual males had hindwing surface loss ($r = .41$, $p = .04$), while in females forewings were more often missing some surface area ($r = .40$, $p = .04$). Crypsis was also associated with more wing tears overall (significant in females with $r = .36$ and $p = .04$) and more bias in wing tears towards hindwings (significant in males with $r = .53$ and $p = .001$; Table 1). Correlations between damage parameters and crypsis were barely affected by accounting for wing length (Appendix S2, Table B2). Species that were thought to rely more on crypsis for defence tended to fly more slowly for their size (PIC on residuals of flight speed over wing length: males $r = -.58$, $p = .005$; females $r = -.35$, $p = .1$). Nevertheless, we found a correlation between wing surface loss and flight speed, but not between wing surface loss and crypsis.

In species in which females showed less foraging activity than males (as indicated by the sex ratio in traps), females had similar wing surface loss overall but more tears in their wings than conspecific males, relative to species with less marked differences in foraging activity levels between the sexes (Table 1). As predicted, species with a larger sex difference in foraging activity also showed

a larger difference in the proportion of symmetrical wing surface loss for both fore- ($r = .75$) and hindwing ($r = .54$) pairs (Figure 5). No correlations with sexual size dimorphism were detected (Table 1).

4 | DISCUSSION

Here we present the first multi-species phylogenetically informed comparative analysis of bodily damage in wild animals, testing hypotheses regarding correlations between prey species traits and damage patterns. We found extensive variation among butterfly species in the extent and symmetry of wing surface loss, as well as degree of bias towards hindwings, while within-species sex differences in damage were modest. Males of faster flying butterflies had less wing surface loss overall (relative to males of slower species); crypsis did not have a major effect on the degree of wing surface loss or the proportion of wing surface loss that is symmetrical; and within species, lower foraging activity levels were associated with a higher proportion of symmetrical wing surface loss.

Our data suggest that damaging attacks on butterflies occur most often when they are in flight. The weak correlation between scale loss and wing surface loss indicates that while scales may be gradually lost with age (Kemp, 2001), wing surface loss is more sporadic, suggesting that wing surface loss is mainly caused by failed predator attacks. Symmetrical wing surface loss is most likely due to instances when butterflies are attacked with their wings closed, as is the case when they are at rest (Shapiro, 1974). Across all species included in this study, most of the observed wing surface loss was non-symmetrical and thus was most likely incurred when wings were open, as is the case during flight. In most species, symmetrical damage was more prevalent in forewings,

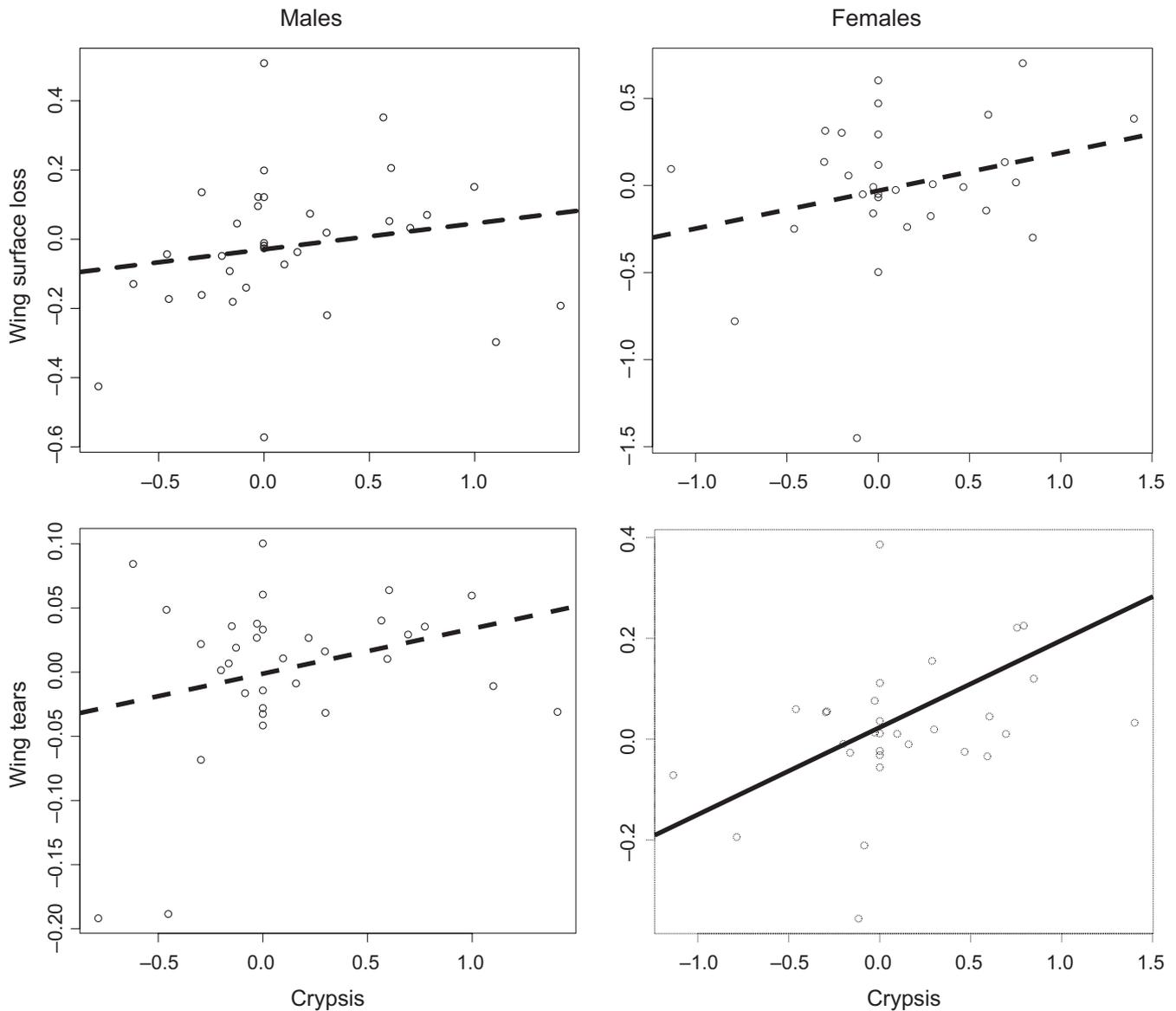


FIGURE 4 Phylogenetic independent contrasts between the estimated importance of crypsis and damage parameters across 34 butterfly species in a forest in Uganda. Significant relationships are depicted with continuous trend lines (statistical results are given in Table 1)

suggesting that attacks on resting butterflies are directed mostly towards forewings. That wing surface loss on one side predicted wing surface loss on the other only for forewings can also be explained by attacks on forewings that are held together. Since geckos predominantly cause symmetrical surface loss in hindwings (Halali et al., 2019), the observed bias in such surface loss towards forewings may indicate that predators with a higher vantage point than lizards may be more frequent attackers of resting butterflies in this system. Furthermore, overall wing damage was more common in hindwings, which could be explained by damage inflicted by predators pursuing butterflies in flight. Thus, both bias of symmetrical wing surface loss to forewings and bias of non-symmetrical wing surface loss to hindwings point towards birds as important butterfly predators in this system. Invertebrates are unlikely to be significant predators of the large butterflies this study relies on (Sang & Teder, 2011) and are unlikely to cause wing damage of the

type recorded in the present study, with the possible exception of mantises.

In species in which females appeared to forage less than males, the proportion of symmetrical wing surface loss was higher in females, suggesting that less active butterflies are more frequently attacked while at rest. However, it is questionable to what extent foraging activity is measured by traps catches represents overall activity which also includes mate finding behaviour and oviposition behaviour (Westerman et al., 2019). One may argue that foraging provides the fuel for flight and thus that more foraging behaviour is correlated with more flight, but this remains to be tested. Furthermore, data on species' activity budgets are required to test whether a lower activity level is correlated with a higher proportion of symmetrical wing surface loss across species as well. Assuming that butterflies fly only a minority of the time, these results may provide further evidence that increased movement is associated with

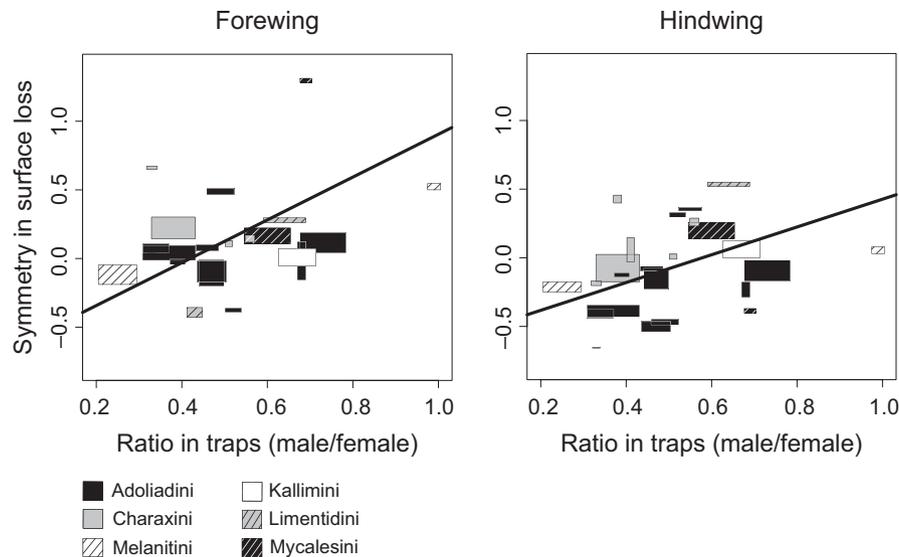


FIGURE 5 Raw correlations between sex differences in foraging activity and sex differences in damage parameters across 34 butterfly species in a forest in Uganda. Solid lines indicate significant relationships. Trend lines are derived from analyses using a restricted maximum likelihood (REML) framework that takes into account phylogenetic non-independence and measurement error (statistical results in Table 1). Clades are pattern-coded, and relative sample size is represented by the respective width and height of markers (within graphs). As the metric of sexual difference, we used the log of the mean values in males divided by that mean in females ($\log(m/f)$)

increased attack risk (Ioannou & Krause, 2009). However, it is also possible that butterflies take flight when they have been detected by predators and thus incur non-symmetrical wing surface loss in flight after being detected at rest.

We did not find any correlations between crypsis and the proportion of symmetrical wing surface loss or the overall wing surface loss, and thus no support for our hypothesis that cryptic species would have a lower frequency of symmetrical damage because they are less frequently spotted while resting with wings closed. Instead, crypsis was significantly associated with other damage parameters that are probably less often the result of failed predator attacks, such as the frequency and position of wing tears. This suggests that crypsis does not confer a lower likelihood of being attacked while at rest, perhaps because of differences in activity patterns among species or because less cryptic species have alternative defences that are similarly effective. For example, in our data, species that relied less on crypsis tended to fly faster.

Males of faster flying butterfly species had on average less wing surface loss. This was significant when tested in isolation (an a priori prediction), but not when Bonferroni–Holm correction was applied to account for testing multiple correlations between flight speed and other damage parameters for which we had no a priori predictions. Among males, faster flying species had on average about 30% less wing surface loss than species that fly half as fast. This was the case despite larger butterflies flying faster on average and larger butterflies having more wing surface loss on average. The lesser extent of wing surface loss in faster butterflies suggests that faster flying butterflies are either more successful at evading predators without being damaged or are attacked less often. It is not likely that faster flight more often leads to death as the outcome of a predator attack. To our knowledge, the detected correlation between flight speed

and wing surface loss provides the first evidence that investment in high flight speed can be an effective alternative to investment in, for example, chemical defence in butterflies, a trade-off suggested by Srygley (1990).

Absence of a correlation between flight speed and wing surface loss in females (in contrast to males) suggests that flight speed is less important as an anti-predator trait for females. In most species in our study, females appear to forage less than males (Figure 5). Moreover, females may rely more on defences other than flight speed, such as mimicry (Ohsaki, 1995; Wallace, 1889). Therefore, it may be that females are not attacked as often in flight as males, and as a result, flight speed may affect damage patterns to a lesser extent in females than in males. Notably, flight performance is also selected for in other contexts, such as mate acquisition (Wickman, 1992), emphasizing that differences in selection on traits between sexes, such as on flight speed, are not only exerted by predators.

Species-specific adult longevity can affect the observed extent of damage, and longevity may be correlated with other species traits, potentially leading to confounded predictors. For example, individuals of faster flying species that are also shorter-lived (Molleman, Ding, Boggs, Carey, & Arlet, 2009a; Molleman et al., 2008; Molleman, Ding, Wang, & Carey, 2009b; Tufto et al., 2012) would have fewer days to accumulate damage. However, such differences in longevity are unlikely to affect the location or symmetry of wing surface loss. Furthermore, distribution of damage in a population depends on the relative rates of different attack outcomes—escape without damage, escape with damage or death (Lima & Dill, 1990; Wourms & Wasserman, 1985)—which could differ systematically among species, sexes and behavioural contexts. While it is not likely that faster flying species have a lower chance of surviving predator attacks, the situation is less clear for crypsis, because crypsis may not provide

any advantage once an individual has been detected. Inevitably, any inference about predation based on damage is less straightforward than direct observations of predation events. Nevertheless, we found interpretable species and sex differences in the extent, location and symmetry of wing damage.

The observed distribution of damage in a population also depends on the effects of damage on survival: when damage reduces life expectancy, lower frequencies of damaged individuals will be encountered. Laboratory experiments demonstrate decreases in life expectancy when legs of insects are amputated (Carey et al., 2009 and references therein), and such effects may be amplified in the wild where animals have to forage, compete and avoid predators (Bulstrode, King, & Roper, 1986; Stirling, 1969). The effect of damage on an individual's life span necessarily depends on the location and extent of damage (Carey et al., 2007). For example, butterflies can fly well without hindwings (but cannot perform evasive manoeuvres), whereas forewing surface loss greatly reduces overall flight performance (Jantzen & Eisner, 2008). Therefore, the prevalence of hindwing surface loss that we observed may in part be due to the less severe survival consequences of hindwing damage compared with forewing damage.

Further studies should evaluate additional traits that might influence wing damage (e.g. palatability, mimicry, wing strength, mate locating tactics, thermoregulation posture, activity budget), for which we did not have sufficient data. Species that may be aposematic (perhaps *E. kakamega*, *E. medon* and *E. harpalyce*), those that are possibly Batesian mimics of aposematic moths (*E. alacris*, *E. zaddachi*, *E. eusemoides*) or those that show marked sexual dimorphism (*E. medon*, *E. kakamega*) did not appear to form outliers in our data (Figure 2; Molleman, Whitaker, & Carey, 2010). Thermoregulation posture probably has a strong phylogenetic signal (Shreeve & Dennis, 1992), as within our dataset basking with open wings was a behaviour mainly observed in Adoliadini butterflies. Perhaps this posture is responsible for a lower proportion of individuals with symmetrical wing surface loss in forewings in this tribe. Palatability could be quantified using assays with ants (Molleman, Kaasik, Whitaker, & Carey, 2012), and wing strength could be assessed using experiments on live butterflies (DeVries, 2002). Further comparative studies could use image analysis methods to quantify damage (Halali et al., 2019) and to measure species traits. For example, for quantification of importance of crypsis we relied only on opinions of humans, which may deviate from the perception of actual predators (Church et al., 1998; Walton & Stevens, 2018). Further insight into how butterfly traits such as activity, flight speed and aposematism (e.g. Westerman et al., 2019) are correlated with each other would also contribute to understanding links between butterfly traits and wing damage patterns. We also note that our results illustrate that it is especially important to use a phylogenetic approach. For example, the *Charaxes* species group is often distinct from other groups (Figure 3) and it is important to ensure that it is not such phylogenetic effects that drive relationships. A phylogenetic comparative approach deals with this by accounting for species interrelatedness.

Our comparative study of damage reveals novel information about predator–prey interactions that are not easily studied directly, such as the effects of foraging activity, crypsis and flight speed on predator attack rates and outcomes in the wild. Specifically, our results provide evidence that damaging attacks tend to occur more often on active rather than resting prey and that fast flight is an effective anti-predator trait, with a doubling of escape speed corresponding to about 30% less wing surface loss. Furthermore, foraging activity levels appear to be more important than crypsis in determining the frequency of attacks on butterflies. These results suggest that butterflies are more often attacked during flight rather than at rest and that flying rapidly is effective for escaping pursuing predators.

ACKNOWLEDGEMENTS

We thank Boniface Balyeganira, Francis Katuramu Kanywanii, Katusabe Swaibu, Mwesige Isaiah, Aliganyira Christopher, Harriet Kesiime, John Koojo, Francis Akoch Edigu, Aisha, Musana Moses and Mary Alum, for their invaluable assistance in the field and in the laboratory; Balyeganira Bonny, Mbabazi Edith and Karoline Zilmer for data entry; and Vivek Philip Cyriac for preparing figures. We are grateful to Torben B. Larsen and Perpetra Akite for offering their expert opinions on butterfly defenses. We thank Tiit Teder, Anu Tiitsaar, Vivek Philip Cyriac and Gopal Murali for conceptual, statistical, textual assistance and tips on literature. Robin van Velzen shared DNA sequence data of *Cymothoe* and provided comments for improvement of the manuscript. Steve Collins and Oskar Brattström helped with butterfly identification. We thank the Uganda Wildlife Authority (U.W.A.) and the Ugandan National Council for Science and Technology (U.N.C.S.T.) for permission to carry out the research. Funding was provided by the National Institute on Aging (PO1 AG022500-01 and PO1 AG608761-10 to JRC), the University of California, Davis (Bixby International Travel Grant to MRLW), the US National Science Foundation (Grant 1309425 to MRLW), the Estonian Science Foundation (grant 9215, group grant IUT20-33), the European Regional Development Fund (Centre of Excellence FIBIR), the Région Bretagne (ACOMB and SAD to AP), the Centre National de la Recherche Scientifique (CNRS; ATIP grant to AP) and the Department of Science and Technology, Government of India (INSPIRE Faculty Award to UK grant number DST/INSPIRE/04/2013/000476).

AUTHORS' CONTRIBUTIONS

F.M. co-designed the study, was responsible for data collection and logistics, data compilation and proofing, and led the analyses and writing. J.J. performed data exploration, including analyses of variance and within-individual correlations. R.B.D. performed the phylogenetic comparative analyses and constructed the figures. M.R.L.W. contributed to data collection, writing style and organization. T.T. was instrumental in directing data analyses and framing of the text. A.P. helped focus the write-up. E.Ö. performed DNA sequencing and constructed the dated phylogeny. N.W. was instrumental for sequencing of butterfly DNA, and assisted with construction of the dated phylogeny. U.K. helped focus the write-up. K.A.-P. sequenced DNA. A.K. advised on all aspects of statistical analyses and

calculation of measurement errors, and implemented the weighted phylogenetic comparative method in R. J.R.C. co-designed the study and helped in framing and illustration. All authors contributed critically to the drafts and gave final approval for publication.

ORCID

Freerk Molleman  <https://orcid.org/0000-0002-6551-266X>

Juhan Javoiš  <https://orcid.org/0000-0003-1283-9666>

Robert B. Davis  <https://orcid.org/0000-0002-0104-0747>

Toomas Tammaru  <https://orcid.org/0000-0002-6892-5910>

Andreas Prinzing  <https://orcid.org/0000-0002-8736-1946>

Niklas Wahlberg  <https://orcid.org/0000-0002-1259-3363>

DATA AVAILABILITY STATEMENT

DNA sequences have been uploaded to GenBank if not already archived there (see Appendix S1 Table A1). The metadata as well as the raw data used in this study are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.r2280gb8j> (Molleman et al., 2019).

REFERENCES

- Aduse-Poku, K., Brakefield, P. M., Wahlberg, N., & Brattström, O. (2017). Expanded molecular phylogeny of the genus *Bicyclus* (Lepidoptera: Nymphalidae) shows the importance of increased sampling for detecting semi-cryptic species and highlights potentials for future studies. *Systematics and Biodiversity*, *15*, 115–130.
- Aduse-Poku, K., Brattström, O., Kodandaramaiah, U., Lees, D. C., Brakefield, P. M., & Wahlberg, N. (2015). Systematics and historical biogeography of the old world butterfly subtribe Mycalesina (Lepidoptera: Nymphalidae: Satyrinae). *BMC Evolutionary Biology*, *15*, 167–170. <https://doi.org/10.1186/s12862-015-0449-3>
- Aduse-Poku, K., Vingerhoedt, E., & Wahlberg, N. (2009). Out-of-Africa again: A phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on five gene regions. *Molecular Phylogenetics and Evolution*, *53*, 463–478. <https://doi.org/10.1016/j.ympev.2009.06.021>
- Alexander, L. G., & DeVries, P. J. (2012). Variation in capture height and trap persistence among three Costa Rican understory butterfly species. *Journal of Tropical Ecology*, *28*, 585–589. <https://doi.org/10.1017/S0266467412000533>
- Arlet, M. E., Carey, J. R., & Molleman, F. (2009). Species, age and sex differences in type and frequencies of injuries and impairments among four arboreal primate species in Kibale National Park, Uganda. *Primates*, *50*, 65–73. <https://doi.org/10.1007/s10329-008-0119-9>
- Bakdash, J. Z., & Marusich, L. R. (2017). Repeated measures correlation. *Frontiers in Psychology*, *8*, 456. <https://doi.org/10.3389/fpsyg.2017.00456>
- Bieber, C., & Ruf, T. (2009). Summer dormancy in edible dormice (*Glis glis*) without energetic constraints. *Naturwissenschaften*, *96*, 165–171. <https://doi.org/10.1007/s00114-008-0471-z>
- Bulstrode, C., King, J., & Roper, B. (1986). What happens to wild animals with broken bones? *The Lancet*, *4*, 29–31. [https://doi.org/10.1016/S0140-6736\(86\)91905-7](https://doi.org/10.1016/S0140-6736(86)91905-7)
- Carey, J. R., Liedo, P., Müller, H.-G., Wang, J.-L., Yang, W., & Molleman, F. (2009). Leg impairments elicit graded and sex-specific demographic responses in the tephritid fruit fly *Anastrepha ludens*. *Experimental Gerontology*, *44*, 541–545. <https://doi.org/10.1016/j.exger.2009.05.006>
- Carey, J. R., Pinter-Wollman, N., Wyman, M., Müller, H.-G., Molleman, F., & Zhang, N. (2007). A search for principles of disability using experimental impairment of *Drosophila melanogaster*. *Experimental Gerontology*, *42*, 166–172. <https://doi.org/10.1016/j.exger.2006.10.002>
- Carpenter, G. D. H. (1941). The relative frequency of beak-marks on butterflies of different edibility to birds. *Proceeding of the Zoological Society London A*, *3*, 223–232.
- Church, S. C., Bennett, A. T. D., Cuthill, I. C., Hunt, S., Hart, N. S., & Partridge, J. C. (1998). Does Lepidopteran larval crypsis extend into the ultraviolet? *Naturwissenschaften*, *85*, 189–192. <https://doi.org/10.1007/s001140050483>
- Davis, R. B., Javoiš, J., Kaasik, A., Öunap, E., & Tammaru, T. (2016). An ordination of life-histories using morphological proxies: Capital vs income breeding in insects. *Ecology*, *99*, 2112–2124. <https://doi.org/10.1002/ecy.1435>
- De Ruiter, L. (1952). Some experiments on the camouflage of stick caterpillars. *Behaviour*, *4*, 222–232.
- DeVries, P. J. D. (2002). Differential wing toughness in distasteful and palatable butterflies: Direct evidence supports unpalatable theory. *Biotropica*, *34*, 176–181.
- Edmunds, M. (1974). Significance of beak marks on butterfly wings. *Oikos*, *25*, 117–118. <https://doi.org/10.2307/3543555>
- Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, *125*, 1–15. <https://doi.org/10.1086/284325>
- Fermon, H., Waltert, M., & Mühlenberg, M. (2003). Movement and vertical stratification of fruit-feeding butterflies in a managed West African rainforest. *Journal of Insect Conservation*, *7*, 7–19.
- Hailman, J. P. (1977). *Optical signals: Animal communication and light*. Bloomington, IN: Indiana University Press.
- Halali, D., Krishna, A., & Kodandaramaiah, U. & Molleman, F. (2019). Lizards as predators of butterflies: shape of wing damage and effects of eyespots. *Journal of the Lepidopterists' Society*, *73*, 78–86.
- Heithaus, M., Frid, A., & Dill, L. (2002). Shark-inflicted injury frequencies, escape ability, and habitat use of green and loggerhead turtles. *Marine Biology*, *140*, 229–236. <https://doi.org/10.1007/s00227-001-0712-6>
- Holm, S., Davis, R. B., Javoiš, J., Öunap, E., Molleman, F., Kaasik, A., & Tammaru, T. (2016). A comparative perspective on longevity: The effect of body size dominates over ecology in moths. *Journal of Evolutionary Biology*, *29*, 2422–2435. <https://doi.org/10.1111/jeb.12966>
- Husak, J. F. (2006). Does speed help you survive? A test with Collared Lizards of different ages. *Functional Ecology*, *20*, 174–179. <https://doi.org/10.1111/j.1365-2435.2006.01069.x>
- Ide, J. Y. (2006). Sexual and seasonal differences in the frequency of beak marks on the wings of two *Lethe* butterflies. *Ecological Research*, *21*, 453–459. <https://doi.org/10.1007/s11284-005-0140-z>
- Ioannou, C. C., & Krause, J. (2009). Interactions between background matching and motion during visual detection can explain why cryptic animals keep still. *Biology Letters*, *5*, 191–193. <https://doi.org/10.1098/rsbl.2008.0758>
- Ives, A. R., Midford, P. E., & Garland, T. (2007). Within-species variation and measurement error in phylogenetic comparative methods. *Systematic Biology*, *56*, 252–270. <https://doi.org/10.1080/10635150701313830>
- Jantzen, B., & Eisner, T. (2008). Hindwings are unnecessary for flight but essential for execution of normal evasive flight in Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 16636–16640.
- Kemp, D. J. (2001). Age-related site fidelity in the territorial butterfly *Hypolimnas bolina* (L.) (Lepidoptera: Nymphalidae). *Australian Journal of Entomology*, *4*, 65–68.
- Kodandaramaiah, U., Lees, D. C., Muller, C. J., Torres, E., Karanth, K. P., & Wahlberg, N. (2010). Phylogenetics and biogeography of a spectacular Old World radiation of butterflies: The subtribe Mycalesina (Lepidoptera: Nymphalidae: Satyrini). *BMC Evolutionary Biology*, *10*, 172. <https://doi.org/10.1186/1471-2148-10-172>
- Kodandaramaiah, U., & Wahlberg, N. (2007). Out-of-Africa origin and dispersal-mediated diversification of the butterfly genus *Junonia*

- (Nymphalidae: Nymphalinae). *Journal of Evolutionary Biology*, 20, 2181–2191. <https://doi.org/10.1111/j.1420-9101.2007.01425.x>
- Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology*, 68, 619–640. <https://doi.org/10.1139/z90-092>
- Molleman, F., Ding, J., Boggs, C. L., Carey, J. R., & Arlet, M. E. (2009a). Does dietary restriction reduce life span in male fruit-feeding butterflies? *Experimental Gerontology*, 44, 601–606. <https://doi.org/10.1016/j.exger.2009.06.008>
- Molleman, F., Ding, J., Wang, J.-L., Brakefield, P. M., Carey, J. R., & Zwaan, B. J. (2008). Adult diet affects life span and reproduction of the fruit-feeding butterfly *Charaxes fulvescens*. *Entomologica Experimentalis Et Applicata*, 129, 54–65.
- Molleman, F., Ding, J., Wang, J.-L., & Carey, J. R. (2009b). Nutrients in fruit increase fertility in wild-caught females of long-lived *Euphaedra* species (Lepidoptera, Nymphalidae). *Journal of Insect Physiology*, 55, 375–383.
- Molleman, F., Javoiš, J., Davis, R. B., Whitaker, M. R. L., Tammaru, T., Prinzing, A., ... Carey, J. R. (2019). Data from: Quantifying the effects of species traits on predation risk in nature: a comparative study of butterfly wing damage. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.r2280gb8j>
- Molleman, F., Kaasik, A., Whitaker, M. R., & Carey, J. R. (2012). Partitioning variation in duration of ant feeding bouts can offer insights into the palatability of insects: Experiments on African fruit-feeding butterflies. *Journal of Research on the Lepidoptera*, 45, 65–75.
- Molleman, F., Kop, A., Brakefield, P. M., DeVries, P. J., & Zwaan, B. J. (2006). Vertical and temporal patterns of biodiversity of fruit-feeding butterflies in a tropical forest in Uganda. *Biodiversity and Conservation*, 15, 107–121. <https://doi.org/10.1007/s10531-004-3955-y>
- Molleman, F., Whitaker, M., & Carey, J. R. (2010). Rating palatability of butterflies using the duration of feeding bouts of ants. *Entomologische Berichten*, 70, 52–56.
- Molleman, F., Zwaan, B. J., Brakefield, P. M., & Carey, J. R. (2007). Extraordinary long life spans in fruit-feeding butterflies can provide window on evolution of life span and aging. *Experimental Gerontology*, 42, 472–482. <https://doi.org/10.1016/j.exger.2007.01.008>
- Monteiro, A., & Pierce, N. E. (2001). Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from COI, COII, and EF-1 alpha gene sequences. *Molecular Phylogenetics and Evolution*, 18, 264–281.
- Ohsaki, N. (1995). Preferential predation of female butterflies and the evolution of Batesian mimicry. *Nature*, 378, 173–175. <https://doi.org/10.1038/378173a0>
- Owen, D. F., & Smith, D. A. (1990). Inter-population variation and selective predation in the meadow brown butterfly, *Maniola jurtina* (L.) (Lepidoptera: Satyridae) in the Canary Islands. *Biological Journal of the Linnean Society*, 39, 251–267. <https://doi.org/10.1111/j.1095-8312.1990.tb00515.x>
- Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290. <https://doi.org/10.1093/bioinformatics/btg412>
- Prudic, K. L., Stoehr, A. M., Wasik, B. R., & Monteiro, A. (2015). Eyespots deflect predator attack increasing fitness and promoting the evolution of phenotypic plasticity. *Proceeding of the Royal Society B*, 282, 20141531. <https://doi.org/10.1098/rspb.2014.1531>
- R Core Team. (2014). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Raffaelli, D. G. (1978). The relationship between shell injuries, shell thickness and habitat characteristics of the intertidal snail *Littorina rudis* Maton. *Journal for Molluscan Studies*, 44, 166–170.
- Rand, A. S. (1954). Variation and predator pressure in an island and a mainland population of lizards. *Copeia*, 4, 260–262. <https://doi.org/10.2307/1440037>
- Rommel, T., Davison, J., & Tammaru, T. (2011). Quantifying predation on folivorous insect larvae: The perspective of life-history evolution. *Biological Journal of the Linnean Society*, 104, 1–18. <https://doi.org/10.1111/j.1095-8312.2011.01721.x>
- Robbins, R. K. (1981). The "false head" hypothesis: Predation and wing pattern variation of Lycaenid butterflies. *The American Naturalist*, 118, 770–775. <https://doi.org/10.1086/283868>
- Ruxton, G. D., Sherratt, T. N., & Speed, M. P. (2004). *Avoiding attack: The evolutionary ecology of crypsis, warning signals, and mimicry*. Oxford, UK: Oxford University Press.
- Sang, A., & Teder, T. (2011). Dragonflies cause spatial and temporal heterogeneity in habitat quality for butterflies. *Insect Conservation and Diversity*, 4, 257–264. <https://doi.org/10.1111/j.1752-4598.2011.00134.x>
- Schoener, T. W. (1979). Inferring the properties of predation and other injury-producing agents from injury frequencies. *Ecology*, 60, 1110–1115. <https://doi.org/10.2307/1936958>
- Shapiro, A. M. (1974). Beak-mark frequency as an index of seasonal predation intensity on common butterflies. *The American Naturalist*, 108, 229–232. <https://doi.org/10.1086/282901>
- Shreeve, T. G., & Dennis, R. L. H. (1992). The development of butterfly settling posture: The role of predators, climate, hostplant-habitat and phylogeny. *Biological Journal of the Linnean Society*, 45, 57–69. <https://doi.org/10.1111/j.1095-8312.1992.tb00631.x>
- Skelly, D. K. (1994). Activity level and the susceptibility of anuran larvae to predation. *Animal Behaviour*, 47, 465–468. <https://doi.org/10.1006/anbe.1994.1063>
- Srygley, R. B. (1990). Associations of butterfly morphology, flight speed and their palatability to the rufous-tailed jacamar. *American Zoologist*, 30, 106–106.
- Stevens, M., Hardman, C. J., & Stubbins, C. L. (2008). Conspicuousness, not eye mimicry, makes "eyespot" effective antipredator signals. *Behavioral Ecology*, 19, 525–531. <https://doi.org/10.1093/beheco/arm162>
- Stevens, M., Searle, W. T. L., Seymour, J. E., Marshall, K. L., & Ruxton, G. D. (2011). Motion dazzle and camouflage as distinct anti-predator defenses. *BMC Biology*, 9, 81. <https://doi.org/10.1186/1741-7007-9-81>
- Stirling, I. (1969). Tooth wear as a mortality factor in the Weddell Seal, *Leptonychotes weddelli*. *Journal of Mammology*, 50, 559–565. <https://doi.org/10.2307/1378783>
- Tonner, M., Novotny, V., Lepš, J., & Komarek, S. (1993). False head wing pattern of the Burmese jungle queen butterfly and the deception of avian predators. *Biotropica*, 25, 474–478. <https://doi.org/10.2307/2388871>
- Torres, C., Osorio-Beristain, M., Mariano, N. A., & Legal, L. (2009). Sex-dependent seasonal feeding activity variations among two species of *Nymphalidae* (Lepidoptera) in the Mexican tropical dry forest. *Annales De La Société Entomologique De France*, 45, 265–274. <https://doi.org/10.1080/00379271.2009.10697610>
- Tufto, J., Lande, R., Ringsby, T. H., Engen, S., Saether, B. E., Walla, T. R., & DeVries, P. J. (2012). Estimating Brownian motion dispersal rate, longevity and population density from spatially explicit mark-recapture data on tropical butterflies. *Journal of Animal Ecology*, 81, 756–769. <https://doi.org/10.1111/j.1365-2656.2012.01963.x>
- Valtonen, A., Molleman, F., Chapman, C. A., Carey, J. R., Ayres, M. P., & Roininen, H. (2013). Tropical phenology: Bi-annual rhythms and inter-annual variation in an Afrotropical butterfly assemblage. *Ecosphere*, 4, 1–15. <https://doi.org/10.1890/ES12-00338.1>
- Van Velzen, R., Bakker, F. T., & van Loon, J. J. A. (2007). DNA barcoding reveals hidden species diversity in *Cymothoe* (Nymphalidae). *Proceeding of the Netherlands Entomological Society Meeting*, 18, 95–103.
- Van Velzen, R., Wahlberg, N., Sosef, M. S., & Bakker, F. T. (2013). Effects of changing climate on species diversification in tropical forest butterflies of the genus *Cymothoe* (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society*, 108, 546–564.
- Vermeij, G. J. (1982). Unsuccessful predation and evolution. *The American Naturalist*, 120, 701–720. <https://doi.org/10.1086/284025>

- Wallace, A. R. (1889). *Darwinism: An exposition of the theory of natural selection with some of its applications* (2nd ed.). London, UK: MacMillan.
- Walton, O. C., & Stevens, M. (2018). Avian vision models and field experiments determine the survival value of peppered moth camouflage. *Communications Biology*, 1, 1–7. <https://doi.org/10.1038/s42003-018-0126-3>
- Westerman, E. L., Antonson, N., Kreutzmann, S., Peterson, A., Pineda, S., Kronforst, M. R., & Olson-Manning, C. F. (2019). Behaviour before beauty: Signal weighting during mate selection in the butterfly *Papilio polytes*. *Ethology*, 00, 1–10.
- Wickman, P. O. (1992). Sexual selection and butterfly design: A comparative study. *Evolution*, 46, 1525–1536. <https://doi.org/10.1111/j.1558-5646.1992.tb01142.x>
- Wourms, M. K., & Wasserman, F. E. (1985). Bird predation on Lepidoptera and the reliability of beak-marks in determining predation pressure. *Journal of the Lepidopterists' Society*, 39, 239–261.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Molleman F, Javoiš J, Davis RB, et al. Quantifying the effects of species traits on predation risk in nature: A comparative study of butterfly wing damage. *J Anim Ecol*. 2020;89:716–729. <https://doi.org/10.1111/1365-2656.13139>