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Differential Wing Toughness in Distasteful and Palatable Butterflies: Direct Evidence Supports Unpalatable Theory¹

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IT IS WELL KNOWN THAT NOT ALL BUTTERFLY SPECIES are equally acceptable food items to bird predators; some are readily eaten, while others may be rejected presumably due to their nasty taste (Poulton 1902; Swynnerton 1915a, b, 1919; Chai 1986). General observations show that palatable butterflies tend to have cryptic coloration and depend upon rapid flight to evade predators, whereas distasteful butterflies minimize predation by advertising noxious qualities associated with a distinctive color pattern and a slow flight (summarized in Turner 1984, 1987; Chai 1986, 1996). These observations are central to understanding the function of butterfly coloration and behavior, learning by butterfly predators, and the theories of unpalatability and mimicry (Marshall 1902; Poulton 1908; Eltringham 1910; Swynnerton 1915a, b, 1919; Carpenter 1932, 1942; Brower 1958a, b; Fisher 1958; Turner 1984; Bowers *et al.* 1985; Chai 1986, 1996).

The basic theory accounting for the evolution of unpalatability and warning coloration requires the differential survival of some individual butterflies following attacks and tasting by predators, and that the experience be memorable to predators (Fisher 1958). A simple extension of unpalatable theory suggests that natural selection should favor aposematic phenotypes possessing a physical toughness that makes them resistant to handling by predators. Indeed, the fact that bodies of unpalatable butterfly taxa tend to be more resilient to handling than palatable ones has long been recognized by entomologists (Poulton 1908, Piepers & Snellen 1909–1918, DeVries 1987). Therefore, toughness appears to be an essential component of butterfly survival from bird attacks; however, the only study of differential toughness among palatable and unpalatable butterflies is that of Carpenter (1941) showing that the number of beak marks on unpalatable museum specimens was significantly greater than palatable ones. From this study, he inferred that unpalatable butterflies surviving bird attacks had tougher wings than palatable ones, thus providing the only empirical evidence in support of toughness as a corollary of unpalatability.

There is a large and diverse body of literature exploring the evolution of butterfly crypsis, unpalatability, mimicry and associated wing traits, and behaviors (Poulton 1902; Blest 1957; Robbins 1981; Turner 1984, 1987; Wourms & Wasserman 1985; Chai 1986, 1996; DeVries 1987; Chai & Srygley 1990; Srygley 1994; Steppan 1996; Beccaloni 1997; DeVries *et al.* 1999; Mallet & Joron 2000 and references therein). Considering the breadth of this literature, it is therefore surprising that differential wing toughness among palatable and unpalatable butterflies has never been measured directly. Accordingly, this study asks whether or not unpalatable butterflies have tougher wings than palatable ones by experimentally estimating the force necessary to tear the wings of representative African butterflies.

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The study was conducted from 12 to 25 September 1999, in the western Usambara Mountains of northeastern Tanzania at the Amani Experimental Field Station (5°15', 38°45'), which has a long history of various human activities (summarized in Iversen 1991). Consequently, the area around Amani is primarily *Maesopsis eminii* Engl. (Rhamnaceae) plantation, second growth forest (invaded extensively by *M. eminii*), other agricultural areas, tea plantations, and associated riparian edges.

Five abundant and widespread nymphalid butterflies were selected to represent palatable or unpalatable species. Palatability was assessed on the basis of their natural history, color pattern, flight behavior, taxonomic affinity, and assessment by natural predators of the five species or their close relatives (Marshall 1902; Swynnerton 1915a, b; Carpenter 1941; Ackery & Vane-Wright 1984; Brower 1984; Turner 1984; Ackery 1987, 1988; Larsen 1991). Based on these criteria, all available evidence suggested that *Junonia terea* Drury (Nymphalinae) and *Bicyclus safitza* Hewitson (Satyrinae) represent palatable species and *Amauris niavius* Linnaeus (Danainae), *Acraea insignis* Distant, and *A. johnstoni* Godman (Acraeinae) represent unpalatable species.

An experimental bird bill was fashioned from a small metal electrical clip (jaws = 10.0 mm long × 3.68 mm wide). A small plastic weighing dish was tied to the end of the clip opposite the jaws with 6 cm of silk thread. This apparatus is hereafter referred to as the clip assembly. A butterfly was killed by a pinch to the thorax, then immediately secured in the jaws of a wooden clothes peg that had been attached to a stiff wire rigidly suspended from the center post below the legs of a photographic tripod. All individuals were secured with the wings closed in a natural resting position such that the clothes peg gripped all four wings. The clip assembly was then carefully attached to the hindwing distal margins of the butterfly such that the jaws gripped the wings between veins Cu₁ and 2A, a position that closely approximates that of beak marks made by birds attacking resting butterflies (Carpenter 1932, 1937, 1938, 1941; DeVries, pers. obs.). The center post of the tripod was then raised slowly until the weighing boat was freely suspended *ca* 20 mm above a receptacle. Once suspended, sufficient dry sand and small metal weights were slowly added to the weighing dish until the entire assembly suddenly tore free of the wing and fell into a collecting receptacle below. The clip assembly and all additional weights were then weighed to the nearest 0.001 g on a model PB53 Mettler-Toledo[®] electronic balance. This weight established the force necessary to tear the clip assembly free of the hindwings, thus providing a measure of relative wing toughness for each individual specimen.

To avoid potential effects of wing condition on measures of relative wing toughness, no individual butterfly tested had any wing damage or faded wing patterns that denoted old age; *i.e.*, all individuals had entire wing margins and were in good condition. Because forewing length is a widely used measure of body size in Lepidoptera (Dudley 2000), the distance from base to apex of one wing was measured with dial calipers to the nearest tenth of a millimeter for all specimens to provide an estimate of body size for each species.

Differences in wing-tear weights and lengths among species were tested using a one-way ANOVA. The potential relationship between tear weight and wing length was tested for each species using linear regression. Significance levels for mean wing tear weight and length in paired species comparisons were adjusted for nonindependence using the sequential Bonferroni-Dunn method (Rice 1989). Wing-tear weights were evaluated using a one-way ANOVA for pooled palatable and unpalatable species.

Mean wing-tear weights differed significantly among the individual species ($F = 141.711$, $P < 0.0001$, $df = 4$), and the ranks by species showed that *A. niavius* had the toughest wings, followed by *A. insignis*, then *A. johnstoni*, while *B. safitza* and *J. terea* tied for the least tough wings (Fig. 1a). Examination of species pairs showed that there were significant wing-tear weight differences among species (Table 1a).

Wing lengths differed among species (Table 1b) and these differences were significant, with the largest species, *A. niavius*, included ($F = 297.303$, $P < 0.0001$, $df = 4$) or excluded from analysis ($F = 17.896$, $P < 0.0001$, $df = 3$). When considered as a group, unpalatable butterflies had greater mean wing lengths than palatable ones, both with *A. niavius* ($F = 31.715$, $P < 0.0001$, $df = 1$) and without it ($F = 30.468$, $P < 0.0001$, $df = 1$). Even though the largest species (*A. niavius*) had the highest tear weight (Fig. 1), regression analysis showed no significant relationship between wing length and tear weight among species; all probability values were between 0.8235 and 0.3397, and all R^2 values were between 0.003 and 0.070.

As a group, unpalatable butterflies had significantly higher wing-tear weights than palatable ones (Fig.

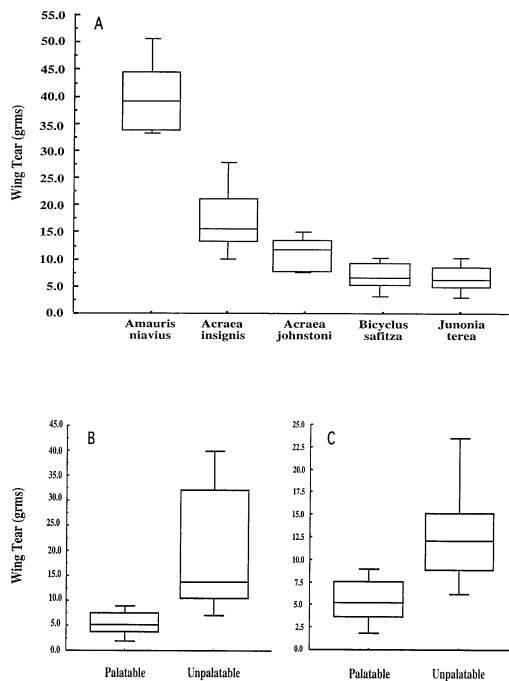


FIGURE 1. Box plot comparisons of wing-tear weights. Each box spans the first to third quartile; the median is shown as the horizontal line within each box; and the vertical bars extend to the maximum and minimum values of the sample. (A) Comparison of wing-tear weights for all species. Samples sizes are as follows: *Amauris niavius* ($N = 13$), *Acraea insignis* ($N = 19$), *Acraea johnstoni* ($N = 15$), *Bicyclus safitza* ($N = 22$), and *Junonia terea* ($N = 18$). (B) Comparison of wing-tear weights of all species grouped by palatable and unpalatable categories. (C) Comparison of wing-tear weights of palatable and unpalatable species excluding the largest species, *A. niavius*.

1b, c), both when the largest species (*A. niavius*) was included ($F = 47.623$, $P < 0.0001$, $df = 1$), and excluded from analysis ($F = 50.201$, $P < 0.001$, $df = 1$). When comparing species pairs, tear weights differed among unpalatable butterflies, but not palatable ones (Table 1a); however, it is of considerable interest that the wing-tear weight of *A. johnstoni* differed from other unpalatable species but not from either palatable species (Table 1a).

Ecological observations and basic unpalatability theory for butterflies suggest that aposematic species should evolve physical attributes that make them resistant to handling by predators (Poulton 1908, Fisher 1958). Using the frequency of beak-marked museum specimens, Carpenter (1941) inferred that unpalatable butterflies were more resistant to bird attacks than palatable ones. The present work, however, provides the first direct experimental evidence that some unpalatable butterfly species have significantly tougher wings than sympatric palatable species (Fig. 1b, c; Table 1a). In concert with Carpenter (1941), this study supports theory by suggesting that a toughened wing integument may be a general trait associated with the evolution of distastefulness in butterflies. The obvious next step will be to test the phylogenetic relationship between these traits through a large comparative study that explores a wide range of butterfly wing lengths, including the effects of flexural stiffness (Steppan 1996).

Birds eat adult butterflies and almost certainly influence the evolution of butterfly wing traits (Poulton 1902; Carpenter 1932, 1937, 1938; Brower 1958a, b; Wourms & Wasserman 1985; Chai 1986, 1996). Two observations make it likely that the experimental methods used in this study simulated wing damage inflicted by a natural bird bill. First, the experimental clip assembly used here approximated the bill sizes of four species of Amani bulbuls known to eat butterflies: *Andropadus gracilis* Cabanis, *A. masukuensis* Shelley, *A. milanensis* Shelley, and *A. nigriceps* Shelley (Pycnonotidae). Second, the form of wing damage inflicted experimentally closely resembled damage known to result from bird attacks in the wild (DeVries, pers. obs.; see also illustrations in Carpenter 1932, 1937).

TABLE 1. (A) Wing-tear differences among species pairs. (B) Wing length differences among species pairs. Bonferroni-Dunn comparisons are significant at $P < 0.005$. Abbreviations: * = significant; NS = not significant.

Comparison	Mean wing tear	Critical difference	P	Significance
(A)				
<i>naivius</i> × <i>insignis</i>	23.266	4.678	<0.0001	*
<i>naivius</i> × <i>johnstoni</i>	29.754	4.924	<0.0001	*
<i>naivius</i> × <i>safitza</i>	33.546	4.546	<0.0001	*
<i>naivius</i> × <i>terea</i>	33.871	4.730	<0.0001	*
<i>insignis</i> × <i>johnstoni</i>	6.488	4.489	<0.0001	*
<i>insignis</i> × <i>safitza</i>	10.280	4.070	<0.0001	*
<i>insignis</i> × <i>terea</i>	10.605	4.274	<0.0001	*
<i>johnstoni</i> × <i>safitza</i>	-3.792	4.351	0.0139	NS
<i>johnstoni</i> × <i>terea</i>	4.117	4.543	0.0106	NS
<i>safitza</i> × <i>terea</i>	0.325	4.130	0.8209	NS
(B)				
<i>naivius</i> × <i>insignis</i>	15.893	1.814	<0.0001	*
<i>naivius</i> × <i>johnstoni</i>	16.995	1.909	<0.0001	*
<i>naivius</i> × <i>safitza</i>	19.743	1.763	<0.0001	*
<i>naivius</i> × <i>terea</i>	17.717	1.834	<0.0001	*
<i>insignis</i> × <i>johnstoni</i>	1.102	1.740	0.0714	NS
<i>insignis</i> × <i>safitza</i>	3.850	1.578	<0.0001	*
<i>insignis</i> × <i>terea</i>	1.824	1.657	0.0021	*
<i>johnstoni</i> × <i>safitza</i>	-2.748	1.687	<0.0001	*
<i>johnstoni</i> × <i>terea</i>	0.722	1.762	0.2403	NS
<i>safitza</i> × <i>terea</i>	-2.026	1.601	0.0005	*

Available evidence suggests that all species of African *Acraea* are unpalatable (Eltringham 1910, 1912; Swynnerton 1915b, 1919; Owen 1971; Nahrstedt & Davis 1981; Pierre 1984). In his review of nymphalid host plants, Ackery (1988) found two major host plant themes that reflect the cladistic analysis of *Acraea* by Pierre (1984)—a general Violaes feeding group and an Urticales feeding group. Although all *Acraea* are suspected to contain cyanide compounds (Owen 1971, Nahrstedt & Davis 1981), Ackery (1988) noted that only species from the Violaes feeding group tested positive for cyanide production; however none from the Urticales group had been examined.

One result from this study suggests that assessing palatability within *Acraea* may be more complex than previously thought. The species examined here represent both feeding groups—*A. insignis* on Violaes and *A. johnstoni* on Urticales. Wing toughness, however, differed between *A. insignis* and *A. johnstoni*, but not between *A. johnstoni* and the palatable species *B. safitza* and *J. terea* (Table 1a). If wing toughness can be used as a measure of relative palatability, then these observations open the possibility of differential palatability among species in the two *Acraea* feeding groups.

Ventral markings at the hindwing distal margin of palatable butterflies, such as the eyespots in the Satyrinae or “false-head” in the Lycaenidae, have evolved to function as targets that divert predator attacks away from vital body areas (Blest 1957, Robbins 1981, Wourms & Wasserman 1985). In essence, the attacked butterfly may escape while the predator is left with only a piece of wing. These observations in combination with the present study provide an impetus for asking if there is a differential toughness between the target area (e.g., eyespots) and other areas of the hindwing in palatable butterflies. Specifically, has natural selection resulted in hindwing target areas that are more fragile than the wing areas surrounding them? This question can be addressed using the methods described here.

In summary, by extending unpalatable theory this study provides a precedent for conducting a large comparative study on differential wing toughness as an evolutionary correlate among many species of palatable and distasteful butterflies. In a similar manner, this study also suggests new ways of assessing palatability among members of *Acraea* and other groups of butterflies that are traditionally considered to be distasteful. Finally, the results here provide a motive and a means for asking whether or not the target patterns on palatable butterflies form the weakest portion of the hindwing.

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