

**Prey mistake masquerading predators for the innocuous items they
resemble**

John Skelhorn

Centre for Behaviour and Evolution, Institute of Neuroscience, Newcastle University,
Henry Wellcome Building, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK

Email: John.Skelhorn@ncl.ac.uk

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1 **MAIN TEXT**

2 Understanding how natural selection has shaped animals' visual appearances to aid
3 predator avoidance and prey capture, has been an ongoing challenge since the
4 conception of evolutionary theory [1,2]. Masquerade, where animals resemble
5 inedible objects common in the local environment (e.g. twigs, leaves, stones), is one
6 of a handful of strategies that has been suggested to serve both protective and
7 aggressive functions (i.e. to work for both prey and predators) [3]. There is now good
8 evidence for protective masquerade: predators detect masquerading prey, but ignore
9 them because they mistake them for the inedible objects they resemble [4].
10 However, there is no evidence that predators can benefit from aggressive
11 masquerade [3,5]. Here, I tested the idea that prey detect masquerading predators
12 but mistake them for the innocuous items that they resemble, making them less wary
13 and easier for predators to catch. Since prey can only mistake masquerading
14 predators for the objects they resemble if they have previous experience of those
15 items, I manipulated House Crickets' *Acheta domesticus* experience with dead
16 leaves, before placing them in tanks with dead-leaf-resembling Ghost mantises
17 *Phyllocrania paradoxa*. I found that mantises given crickets with experience of
18 unmanipulated dead leaves, caught crickets faster and after fewer attempts, than
19 mantises given crickets without experience of dead leaves, or crickets with
20 experience of manipulated dead leaves that no longer resembled mantises. This
21 clearly shows that predators can benefit from aggressive masquerade.

22 Since Ghost mantises appear to masquerade as dead leaves, I manipulated
23 the crickets' experience with dead leaves from plants found in the same geographic
24 area as the mantises *Coffea liberica* and *Ptilostigma thonningii*. I divided the leaf-
25 naïve crickets into 3 equally-sized experimental groups, and gave individuals from

26 each group a series of four experience manipulation trials. During these trials,
27 individual crickets were placed in an arena where they were allowed to interact with
28 either unmanipulated dead leaves, manipulated dead leaves (covered in circular
29 white stickers) that no longer resembled mantises, or an empty arena depending on
30 their experimental group (See supplemental material). Crickets were initially wary of
31 dead leaves but their latency to touch the leaves declined across trials (see
32 supplemental material and Fig S1) indicating they learned that the leaves did not
33 pose a risk. Furthermore, there was no difference in the time to approach
34 manipulated and unmanipulated leaves (see supplemental material and Fig S1)
35 indicating that manipulation did not influence the risk crickets perceived leaves to
36 pose.

37 I then presented each of the crickets to one of thirty-six mantises, and
38 monitored the time taken for the mantis to catch the cricket and the number of times
39 the mantis struck at the cricket but failed to catch it (see ESM). Both of these
40 measures differed among the three experimental groups (Latency to catch, Welch's
41 ANOVA $F_{2,18.06}=26.569$, $P<0.001$. Failed attempts, Kruskal Wallis test; $X^2 = 22.172$,
42 $P<0.701$, $df=2$; Fig 1). I found no significant differences in the time (Welch's ANOVA
43 $F_{1,21.298}=0.541$, $P=0.470$; see Fig 1A), or number of attacks (Kruskal Wallis test; $X^2 =$
44 2.787 , $P=0.095$, $df=1$; see Fig 1B), it took mantises to catch crickets that had
45 experienced manipulated dead leaves compared to crickets that had experienced an
46 empty arena, confirming that manipulating leaves successfully destroyed their
47 resemblance to mantises. But crucially, I found that mantises caught crickets that
48 had experienced unmanipulated dead leaves significantly faster (Welch's ANOVA
49 $F_{1,32.537}=55.757$, $P<0.001$; see Figs 1A), and after significantly fewer failed attacks
50 (Kruskal Wallis test; $X^2 = 20.707$, $P<0.001$, $df=1$; see Figs 1B), than crickets that had

51 experienced manipulated dead leaves or an empty arena. Crickets with experience
52 of unmanipulated leaves mistook the mantises for the dead leaves they had learned
53 were innocuous, and were therefore easier for the mantises to catch than crickets
54 that had no experience with leaves that resembled the mantises.

55 My results suggest that masquerade has benefits above and beyond deterring
56 predators. It can enhance prey capture by causing prey to mistake masquerading
57 predators for the innocuous objects they resemble. Protective masquerade has been
58 shown to have important effects on almost all aspects of preys' lives, from habitat
59 selection and feeding decisions [6,7] through to optimal investment in parental care
60 [8]. The efficacy of protective masquerade is also density dependent which likely has
61 important consequences for the evolutionary and population dynamics of
62 masquerading prey [6]. It therefore seems reasonable to predict that similar effects
63 will be seen in masquerading predators (although the effects of density may be
64 subtly different given that prey that misclassify masquerading predators are often
65 killed whilst predators that misclassify masquerading prey are not), and appreciating
66 this will allow us to better understand the behaviour, ecology and evolution of such
67 species. Furthermore, since many masquerading species are both predators and
68 prey, masquerade has the potential to become an important paradigm for future
69 investigations into how selection from predators and prey interact to influence
70 animals' appearances.

71 My results also demonstrate that prey cognition is an important selection
72 pressure driving the evolution of prey capture adaptations. Predator cognition has
73 long been recognized as an important selective pressure driving the evolution of
74 animals' visual appearances [9], but research into prey cognition has received much
75 less attention. Moreover, recent work has highlighted that classic examples of prey

76 capture adaptations thought to exploit prey cognition, may not actually do so. Work
77 on aggressive mimicry (where predators resemble a resource that is important to
78 prey) in Orchid mantises, indicates that mantises appear to exploit the sensory
79 biases of pollinators rather than what they have learned about the flowers of a
80 particular species of orchid [10]. The results I present here suggest that explanations
81 based on sensory processes cannot explain the evolution of all adaptive
82 resemblances in predators and add weight to the argument that we need a better
83 understanding of prey cognition in order to appreciate why predators look the way
84 they do.

85

86 **ACKNOWLEDGEMENTS**

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88 leaves were kindly provided by the *Eden Project*, Cornwall. U.K. The work complied
89 with ASAB guidelines for the of animals in research and teaching.

90

91

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113 **Figure Legends**

114

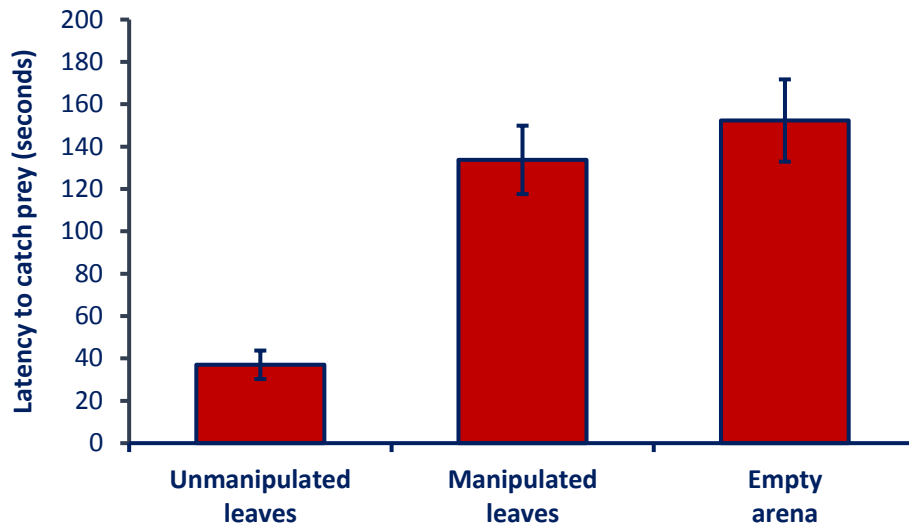
115 Figure 1

116 (A) The latency in seconds (mean +/- SE) to catch the cricket, and (B) the number of
117 failed attacks (mean +/- SE) before the cricket was caught, in the test trial for each
118 experimental group (N = 12 for each group).

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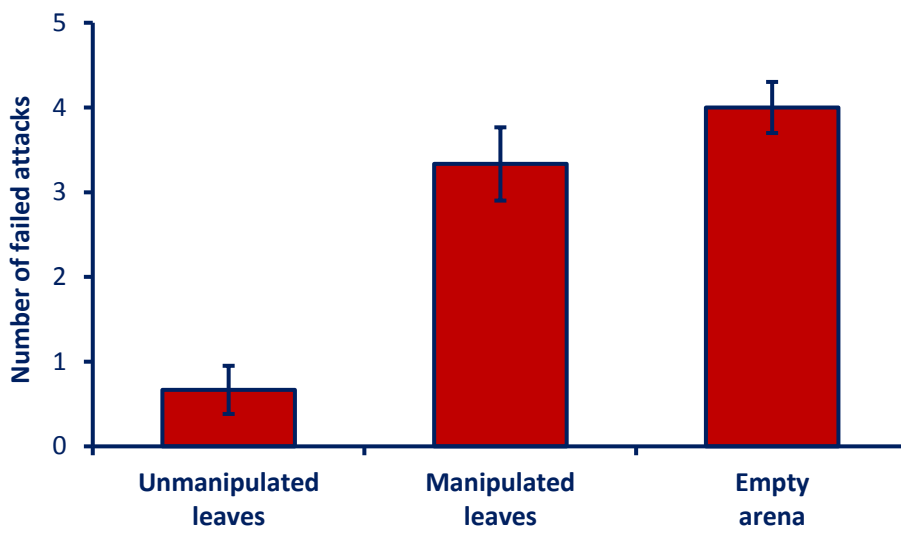
121 **Figure 1A**



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124 **Figure 1B**



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128 **SUPPLEMENTAL MATERIAL**

129

130 **EXPERIMENTAL PROCEDURES**

131 ***Predators***

132 Thirty-six newly-hatched Ghost Mantises (18 male and 18 female) were purchased
133 from an internet auction site. They were housed individually, in a laboratory
134 maintained at 20-23°C and lit by natural daylight. Mantises were initially housed in
135 clear plastic drinking cups covered with a muslin lid. Upon reaching instar 3, they
136 were moved into ventilated plastic tank measuring 37 X 22 x 24 cm (L x W x H). A
137 ball of cotton wool soaked in water was placed in each cup/tank to maintain
138 appropriate humidity levels and to serve as a water source; and a piece of wooden
139 dowel provided mantises with a perch. Mantises were initially fed on flightless fruit
140 flies (*Drosophila melanogaster*), and as they increased in size they were switched to
141 House Crickets. The size of the crickets presented was commensurate with the size
142 of the mantis: when mantises reached their final instar, the point at which they were
143 used in experimental trials, they were eating final instar crickets. I ensured that
144 mantises ate equal numbers of crickets that had, and had not, encountered dead
145 leaves (see below) throughout their development. This was to ensure that any
146 differences in mantis' behaviour observed in the test trial, could not be explained by
147 differences in their familiarity of leaf-naïve compared to leaf-experienced crickets. I
148 achieved this by presenting these prey types alternatively, except when a prey item
149 was left uneaten, at which point mantises were presented with the same prey type in
150 the subsequent presentation.

151

152 **Prey**

153 Newly-hatched House crickets were purchased from commercial live food suppliers
154 throughout the experiment. These were maintained in plastic tanks measuring 37 X
155 22 x 24 cm until they reached the required size. All tanks contained an empty egg
156 carton to provide shelter, and a Petri dish containing a commercial cricket diet mixed
157 with water. Half of the tanks also contained four dead leaves, or pieces of dead
158 leaves, similar in size to adult Ghost mantises. These came from two plant species
159 found in the same geographic area as mantises: *Coffea liberica* and *Piliostigma*
160 *thonningii*, and were kindly provided by the *Eden Project*, Cornwall, U.K.. This
161 effectively created groups of crickets that had, and had not, encountered dead
162 leaves. These were fed to mantises throughout their development (as outlined
163 above), and thirty-six final instar crickets that had not previously encountered dead
164 leaves were used as experimental subjects.

165

166 **Experience manipulation trials**

167 The thirty-six final instar crickets that had not previously encountered dead leaves
168 were randomly allocated to three experimental groups. Each cricket underwent 4
169 experience manipulation trials that were equally-spaced throughout the day before
170 the test trial. Each trial consisted of placing the cricket in a plastic tank measuring
171 (37 X 22 x 24 cm) for a period of 15 minutes. The contents of the tank differed
172 between experimental groups: crickets in the 'Unmanipulated Leaves Group'
173 encountered a piece of dead leaf similar in size to an adult ghost mantis; crickets in

174 the 'Manipulated Leaves Group' encountered a similar leaf with four circular white
175 stickers (8mm in diameter) attached to it in order to alter its visual appearance whilst
176 minimising changes to its structure and odour; and crickets in the 'Empty Arena
177 Group', encountered an empty tank. In each trial, the time taken for the cricket to
178 touch one of the leaves was measured (except in the empty arena group, where
179 there were no leaves present).

180

181 ***Test trial***

182 I presented each of the crickets to a different mantis (ensuring there were equal
183 numbers of male and female mantises in each experimental group), and recorded
184 both the time taken for the cricket to be caught, and the number of times the mantis
185 struck at the cricket but failed to catch it. The trial was performed in the mantis's
186 home tank, and the mantis was food deprived for 7 days before the trial in order to
187 ensure it was motivated to eat. Crickets were introduced via a small hole in the tank
188 wall, that was usually plugged. This was located in one of the two shorter walls, and
189 was positioned centrally on the horizontal axis but just 2 cm from the base of the
190 tank. At the start of the experiment, mantises were located on their dowel perch
191 which was positioned 20 cm from the hole through which crickets were introduced.
192 Once the cricket was introduced the experimenter moved behind a curtain and
193 watched the trial via a live video link. The experimenter was blind to crickets'
194 previous experience and recorded both the time it took for the mantis to catch the
195 cricket, and the number of failed strikes before the cricket was caught. Strikes are
196 unambiguous and involve the mantis lunging forward and extending its forelimbs.

197

198 **Statistical analysis**

199 Experience manipulation trials

200 I ran a repeated measures ANOVA with trial as a within subject factor and
201 experimental group as a between subject factor in order to determine whether the
202 time taken for crickets to touch the dead leaves differed across trials and between
203 the experimental groups. The assumptions of repeated measures ANOVA were met:
204 in each of the trials, the unstandardized residuals were normally distributed (Shapiro-
205 Wilks tests: Trial 1, $W=0.952$, $df=24$, $P=0.300$; Trial 2, $W=0.965$, $df=24$, $P=0.535$;
206 Trial 3, $W=0.968$, $df=24$, $P=0.627$; Trial 4, $W=0.947$, $df=24$, $P=0.237$), the observed
207 covariance matrices of the dependent variables were equal across groups (Box's
208 test, $M=738$, $P=0.571$); and tests for sphericity (Mauchly's test $W=0.694$, $df=5$,
209 $P=0.182$) and homoscedasticity (Levine's tests: Trial 1, $F_{1,22}=0.500$, $P=0.487$; Trial 2,
210 $F_{1,22}=0.604$, $P=0.445$; Trial 3, $F_{1,22}=0.009$, $P=0.924$; Trial 4, $F_{1,22}=0.803$, $P=0.380$)
211 were not significant. I found a main effect of trial ($F=66.113$, $df=3$, $P<0.001$), but
212 there was no effect of experimental group ($F=0.138$, $df=1$, $P=0.714$), and no trial x
213 group interaction ($F=0.694$, $df=3$, $P=0.559$). I then used Bonferroni tests to determine
214 how the latency for crickets to touch the leaves differed across trials. I performed all
215 pairwise comparisons, and found that crickets took significantly longer to touch
216 leaves in trial 1 than in trials 2 ($P<0.001$), 3 ($P<0.001$) and 4 ($P<0.001$). However,
217 latencies didn't differ significantly among trials 2, 3 and 4 (trial 2 Vs 3, $P=0.148$; Trial
218 2 Vs 4, $P=0.920$; Trial 3 Vs 4, $P=1.00$). Taken together these finding indicate that
219 crickets quickly learned that dead leaves were innocuous, and that neither the initial
220 latency to touch the leaves nor the speed at which crickets learned they were
221 innocuous were influenced by whether or not the leaves were manipulated.

222

223 Test trial

224 I used Welch's ANOVA's to test my predictions about the latency to catch crickets
225 because the unstandardized residuals for this measure were normally distributed
226 (Shapiro-Wilks tests, $W=0.946$, $df=36$, $P=0.076$), but within group variances
227 exhibited heteroscedastisity (Levine's tests, $F_{2,33}=3.464$, $P=0.043$). In contrast, I
228 used Kruskal Wallis tests to test my predictions about the number of failed attacks
229 before crickets were caught because the unstandardized residuals for this measure
230 were not normally distributed (Shapiro-Wilks tests, $W=0.926$, $df=36$, $P=0.019$), but
231 within group variances exhibited homoscedastisity (Levine's tests, $F_{2,33}=1.063$,
232 $P=0.357$). My planned comparisons tested the following a priori predictions:

233 (1) That mantises given leaf-naïve crickets would catch crickets more quickly and
234 after fewer failed attempts, than mantises in the other two groups.

235 (2) That mantises given crickets with experience of manipulated dead leaves and
236 mantises given crickets with experience of an empty arena would not differ in
237 either the latency to catch crickets or the number of failed attempts before
238 they caught them.

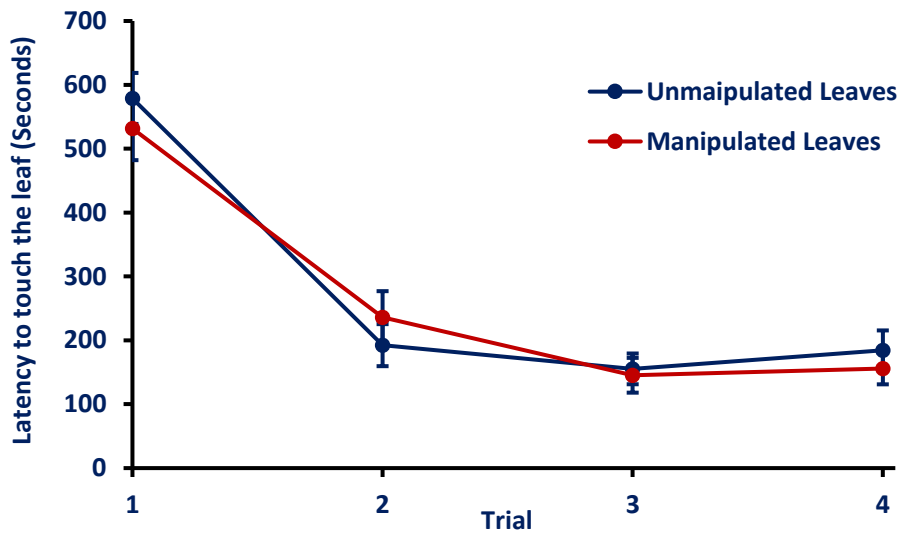
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240 Since I tested only two of planned comparisons for each dependent variable, I did
241 not correct for multiple comparisons. However, doing so would have had no effect on
242 my conclusions: Bonferroni correction would have changed the threshold for a
243 significant P value from 0.05 to 0.025, meaning all the tests currently reported as
244 significant would remain significant. All analyses were conducted using *IBM SPSS*
245 *Statistics 24*.

246 Figure S1

247 The latency in seconds (mean +/- SE) to touch the training stimulus (leaves or
248 manipulated leaves) in each of the four experience manipulation trials for each
249 experimental group (N = 12 for each group).

250



251

252