trypsinization for serial subcultivation (*14*). Membrane changes that are later reflected in changes in intracellular activity may be brought about by trypsinization. Exposure of cells to some substance (or substances) in either the medium or serum during subcultivation may also be a factor. Additionally, the possibility that this differentiation is a "cell aging" phenomenon must be considered. Further studies involving tumorigenicity, cytogenetics, and cloning will be required to more completely define the nature of this phenotypic alteration of C6 glial cells.

De Vellis and associates (*15*) have commonly used RG C6 cells of less than 25 passages for their studies of steroid hormone induction of GPDH, another enzyme marker for oligodendrocytes. It would be of interest to know whether GPDH is present at later cell passages.

The finding of marked GS activity as a factor of passage of cells may explain why little GS was detected previously by Nicklas and Browning (*16*). They reported activities that were 50- to 100-fold lower than those found in whole rat brain. In the present study, the activity of cells at 82 passages and 15 days in culture (2.137 ± 0.096) is almost as high as activities found in whole rat brain (2.940 ± 0.105). To a minor extent the present higher values may also be due to methodology. The higher amount of ATP used in our assay media results in approximately 40 percent increased activity; trypsinization of the cells causes a twofold increase in activity. Finally, this study strongly emphasizes the large amounts of GS present in C6 cells and furthermore stresses the importance of reporting passage when describing properties of C6 glial cells.

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**References and Notes**

1. S. E. Pfeiffer, B. Betschart, J. Cook, P. Mancini, R. Morris, in *Cell, Tissue and Organ Cultures in Neurobiology*, S. Fedoroff and L. Hertz, Eds. (Academic Press, New York, 1977), p. 287; A. Vernadakis and B. Culver, in *The Biochemistry of Brain*, S Kumar, Ed. (Pergamon, in press).
2. P. Benda, J. Lightbody, G. Sato, L. Levine, W. Sweet, *Science* **161**, 370 (1968); P. Benda, K. Someda, J. Messer, W. H. Sweet, *J. Neurosurg.* **34**, 310 (1971); L. J. Embree, H. H. Hess, H. M. Shein, *Exp. Neurol.* **31**, 383 (1971).
3. S. W. Silbert and M. N. Goldstein, *Cancer Res.* **32**, 1422 (1971).
4. M. G. Bissell, L. J. Rubinstein, A. Bignami, M. M. Herman, *Brain Res.* **82**, 77 (1974).
5. J. P. Zanetta, P. Benda, G. Gombos, I. G. Morgan, *J. Neurochem.* **19**, 881 (1972).
6. J. F. McGinnis and J. de Vellis, *Nature (London)* **250**, 422 (1974).
7. M. D. Norenberg and A. Martinez-Hernandez, *Brain Res.* **161**, 303 (1979); S. E. Poduslo and W. T. Norton, *J. Neurochem.* **19**, 727 (1972).
8. O. J. Pritchard, R. M. Clayton, D. I. de Pomerat, *J. Embryol. Exp. Morphol.* **48**, 1 (1978).
9. Obtained through the courtesy of Dr. Jean de Vellis, University of California, Los Angeles.
10. J. R. Prohaska, D. A. Clark, W. W. Wells, *Anal. Biochem.* **56**, 275 (1973).
11. S. Berl, *Biochemistry* **5**, 916 (1966).
12. L. Hertz, personal communication.
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
14. H. Waters and R. L. Walford, *Proc. Soc. Exp. Biol. Med.* **137**, 523 (1971); A. Maizel, C. Nicoline, R. Beserga, *J. Cell. Physiol.* **86**, 71 (1975).
15. G. A. M. Breen, J. F. McGinnis, J. de Vellis, *J. Biol. Chem.* **253**, 2554 (1978); P. Leveille, J. de Vellis, D. S. Maxwell, *Trans. Soc. Neurosci.* **7**, 33 (1977).
16. W. J. Nicklas and E. T. Browning, *J. Neurochem.* **30**, 955 (1978).
17. Partially supported by PHS training grant T32 HD 07072, a Department of Psychiatry developmental psychobiology research group grant, research scientist career development award KO2 MH 42479 (A.V.), and the Medical Research Service of the Veterans Administration. M.D.N. is a Clinical Investigator of the Veterans Administration. We thank K. Mailloux and K. P. Bell for technical assistance.

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## Differential Avoidance of Mimetic Salamanders by Free-Ranging Birds

Abstract. *Members of a free-ranging avian community avoided the mimetic morph of the salamander* Plethodon cinereus *significantly more often than a nonmimetic morph when offered with the model eft stage of* Notophthalmus viridescens *and the palatable salamander* Desmognathus ochrophaeus. *This is apparently the first demonstration of the efficacy of mimetic coloration of salamanders to uncaged birds.*

Although the efficacy of mimicry in salamanders has been demonstrated with caged birds, there have been no attempts to demonstrate the advantage of mimetic coloration to free-ranging birds (*1*). Attempts have been made to assess the value of mimetic coloration of moths and butterflies to wild bird communities by releasing and retrieving individuals painted to resemble palatable or unpalatable species (*2*). None of these studies included the use of unaltered mimics or restricted predation to birds, which are generally assumed to be responsible for the evolution of mimetic colorations.

In our study, we tested the survival value of a presumed mimetic color morph of the polymorphic salamander *Plethodon cinereus*. Certain populations of *P. cinereus* have two color morphs: a red-striped morph, which is present in most portions of the species' range, and an all-red morph, which is nearly uniformly red-orange (*3*). This all-red morph is thought to be a mimic of the eft stage of *Notophthalmus viridescens* that is toxic, aposematic, and a model for other mimic salamanders (*1, 4*).

In order to test the survival value of natural mimics to natural avian communities we have developed an experimental design exposing unaltered salamanders to predation only by birds. Single salamanders were placed in 50 trays 30 cm square with an aluminum wall 10 cm tall; the top of the aluminum was bent inward, forming a 3-cm lip to prevent the escape of the salamanders (*5*). Each tray was nailed to a vertical section of pine log, 60 cm in length and 15 to 20 cm in diameter. Each tray contained four dead maple or oak leaves (or both) from the adjacent deciduous forest; the leaves were soaked with water just prior to each trial. The trays were placed in three rows of 18, 17, and 15 trays; the rows and trays within a row were 10 m apart. The rows of trays were positioned between rows of trees in a 50-year-old pine plantation.

The distribution of salamanders within this grid was randomized from a hypothetical community of a million animals made up of 30 percent *N. viridescens* efts, 40 percent *Desmognathus ochrophaeus*, 24 percent striped *P. cinereus*, and 6 percent all-red *P. cinereus*; a computer generated random orders of contact for 50 individuals from this community. In each trial, an independent random distribution of salamanders was used and the actual percentages varied slightly from the hypothetical community as follows: *N. viridescens*, 29.4 percent; *D. ochrophaeus*, 41.4 percent; striped *P. cinereus*, 23.3 percent; and all-red *P. cinereus*, 5.8 percent (*6*). The *D. ochrophaeus* were offered as alternate, palatable prey because mimicry is effective only when an alternate food source is available (*7*).

Initially each trial lasted for 4 hours (5 to 9 a.m. and 5 to 9 p.m.); the predation rate during five 4-hour trials was 75.1 percent of all edible salamanders (*D. ochrophaeus* and *P. cinereus*). Because of this high predation rate, the length of


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Table 1. Differential survival of salamanders exposed to free-ranging birds.

| Trials | Number offered (percentage of survival) | | | |
|---|---|---|---|---|
| | *Notophthalmus viridescens* | *Desmognathus ochrophaeus* | *Plethodon cinereus* | |
| | | | Striped | All-red |
| 4-hour | 73 (95.9) | 97 (30.9) | 60 (15.0) | 20 (25.0)* |
| 2-hour | | | | |
| First seven | 106 (99.0) | 145 (53.1) | 87 (47.1) | 12 (50.0)* |
| Second seven | 100 (99.0) | 145 (42.1) | 76 (31.6) | 29 (58.6)† |
| All 2-hour | 206 (99.0) | 290 (47.6) | 163 (39.9) | 41 (56.1)‡ |

*Not significantly higher than for striped: 4-hour trials, $\chi^2 = 1.039$; first seven 2-hour trials, $\chi^2 = 0.035$. †Higher than for striped: $\chi^2 = 6.449$, $.01 < P < .025$. ‡Higher than for striped: $\chi^2 = 3.584$, $.05 < P < .1$.

each trial was reduced to 2 hours (7 to 9 a.m. and 5 to 7 p.m.). In all, 950 salamanders were used. Results were recorded as: salamander removed from tray (we assume these did not survive) or salamander remained in tray (survived). From these data, it is possible to contrast the survivorship between species and between the two color morphs of *P. cinereus*. Since no species or morph survived significantly more often during the morning or afternoon trials we have combined these data.

The efts of *N. viridescens* survived far more often than those of the other species; birds removed only 2 of 206 during the 2-hour trials (Table 1). This result was expected because efts are toxic, bright orange-red in coloration, and avoided by birds (*1, 4*). *Desmognathus ochrophaeus* survived slightly more often than the striped *P. cinereus* ($\chi^2 = 2.996$, $.05 < P < .1$); this difference was not significant when analyzed for either the first seven or last seven 2-hour trials (Table 1).

All-red *P. cinereus* survived significantly more often than striped *P. cinereus* when all 2-hour trials or only the last seven are considered (Table 1); this is also true when the last five, six, or eight trials are considered (*8*). There is no difference in survival when only the first seven 2-hour trials are contrasted, suggesting that the all-red morph of *P. cinereus* survived in later trials because birds learned not to take red salamanders. Since both the striped and all-red morphs of *P. cinereus* were from the same localities, we assume that they are equally palatable and that any difference in survival is due to their different coloration.

Birds were seen (as salamanders were being placed into the trays) during 14 of the trials in or near trays (16 robins, 1 catbird). All birds seen in or near trays were adults; one fledgling was seen being fed by an adult during the last trial, but not near any tray. Bird droppings were seen in an additional 48 trays as they were checked at the end of each test period. Leaves had been thrown from trays, ripped, or greatly moved around in 149 of 700 trays during the 2-hour trials. This could only have been the result of bird activity in the tray (*5*). The percentage of trays in which leaves had been moved did not differ between salamanders (*9*).

By contrasting the survival or removal of salamanders only from trays where leaves had been moved, it is possible to test for the survival value of the presumed mimetic coloration with certainty that the mimics had been contacted by avian predators. When leaves had been moved the all-red morph of *P. cinereus* survived 66.7 percent of the time in contrast to the striped morph which survived 20.5 percent of the time (Fig. 1); this difference is significant ($\chi^2 = 7.540$, $.005 < P < .01$). Striped *P. cinereus* and *D. ochrophaeus* survived at the same rate, and there were no differences between these and all-red morphs when leaves had not been moved (Fig. 1).

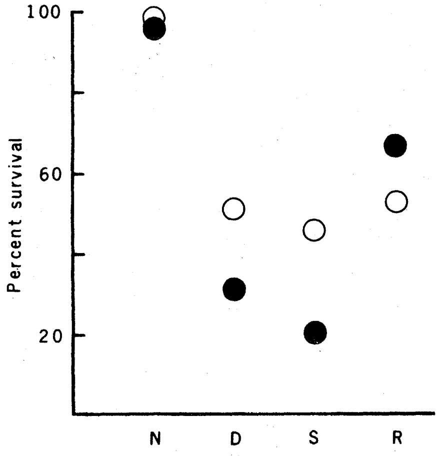


Fig. 1. Survival of salamanders as related to movement of leaves in test trays by birds. Closed circles represent survival in trays where leaves had been moved. Open circles represent survival in trays where leaves had not been moved. Abbreviations: *N*, the model, eft *Notophthalmus viridescens*; *D*, *Desmognathus ochrophaeus*; *S*, striped *Plethodon cinereus*; and *R*, the mimic, all-red *P. cinereus*. The mimics survived significantly more often than the nonmimetic striped *P. cinereus* when leaves had been moved ($\chi^2 = 7.540$, $.005 < P < .01$).

We can conclude that free-ranging bird communities avoid model and mimic salamanders while eating nonmimic salamanders and that the all-red morph of *P. cinereus* is a mimic of eft *N. viridescens*. Mimetic individuals (at a frequency of 20 percent) of a polymorphic species had a great survival advantage over nonmimetic individuals. Further utilization of our experimental design should allow a more natural demonstration of the precepts of mimicry but should be used to augment rather than replace studies with caged avian predators.

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**References and Notes**

1. R. R. Howard and E. D. Brodie, Jr., *Nature (London)* **233**, 277 (1971); *Herpetologica* **29**, 33 (1973); E. D. Brodie, Jr., and R. R. Howard, *Am. Midl. Nat.* **90**, 38 (1973).
2. L. P. Brower, L. M. Cook, H. J. Croze, *Evolution* **21**, 11 (1967); L. M. Cook, L. P. Brower, J. Alcock, *ibid.* **23**, 339 (1969); W. W. Benson, *Science* **176**, 936 (1972); G. P. Waldbauer and J. G. Sternburg, *Evolution* **29**, 650 (1976); M. R. Jeffords, J. G. Sternburg, G. P. Waldbauer, *ibid.* **33**, 275 (1979).
3. The all-red morph of *P. cinereus* is found only in the northeastern United States at frequencies up to 20 percent (S. G. Tilley, personal communication). E. Mathews, *Copeia* (1952), p. 277; C. F. Reed, *ibid.* (1955), p. 253; G. R. Thurow, *Evolution* **15**, 281 (1961); R. Highton, *Bull. Fl. State Mus. Biol. Ser.* **6**, 235 (1962).
4. E. D. Brodie, Jr., *Am. Midl. Nat.* **80**, 276 (1968); ______, J. L. Hensel, Jr., J. A. Johnson, *Copeia* (1974), p. 506.
5. The effectiveness of these trays was demonstrated 10 days after the termination of our tests. Birds had apparently left the pine woods, and all salamanders remained in the trays during three 2-hour tests (150 animals). No leaves were moved.
6. Salamanders from the following localities were used: *N. viridescens* efts, near Rensselaerville, Albany County, New York; *D. ochrophaeus* adults, Albany and Ulster counties, New York; striped and all-red *P. cinereus* adults, Franklin County, Massachusetts. The test grid was located on the E. N. Huyck Preserve, Rensselaerville; *N. viridescens*, *D. ochrophaeus*, and striped *P. cinereus* are native. *Desmognathus ochrophaeus* with red coloration were not used.
7. E. B. Poulton, *The Colours of Animals* (Kegan Paul, Trench, Trübner, London, 1890); H. B. Cott, *Adaptive Coloration in Animals* (Methuen, London, 1957); R. A. Fisher, *The Genetical Theory of Natural Selection* (Dover, New York, 1958).
8. All-red *P. cinereus* survived more often than striped *P. cinereus* during the last five trials ($\chi^2 = 4.828$, $.025 < P < .05$), the last six trials ($\chi^2 = 9.204$, $.001 < P < .005$), and the last eight trials ($\chi^2 = 5.096$, $.01 < P < .025$).
9. The following percentages of trays had leaves moved: *N. viridescens*, 22.8; *D. ochrophaeus*, 18.6; striped *P. cinereus*, 23.9; and all-red *P. cinereus*, 22.0. No pairs of values are significantly different by chi-square analysis.
10. We thank the E. N. Huyck Preserve and its director R. C. Dalgleish for support. S. G. Tilley pointed out this case of mimicry and helped us obtain specimens. R. G. Jaeger, D. R. Formanowicz, Jr., and P. K. Ducey helped formulate the experimental design, and the last two with J. C. Bodenweiser, M. L. Manis, and M. S. Bobka helped with collection of animals and data.

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