doi:10.1111/j.1558-5646.2010.01137.x

POSITIVE FREQUENCY-DEPENDENT SELECTION ON WARNING COLOR IN ALPINE LEAF BEETLES

Matthias Borer,[1,2] Tom van Noort,[1,2] Martine Rahier,[1] and Russell E. Naisbit[1,3,4]

[1]*Laboratory of Evolutionary Entomology, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland*

[3]*Unit of Ecology and Evolution, Department of Biology, University of Fribourg, Chemin du Musée 10, 1700 Fribourg, Switzerland*

[4]*E-mail: russell.naisbit@gmail.com*

Received April 8, 2010
Accepted September 11, 2010

Müller's theory of warning color and mimicry, despite forming a textbook example of frequency-dependent selection, has rarely been demonstrated in the wild. This may be largely due to the practical and statistical difficulties of measuring natural selection on mobile prey species. Here we demonstrate that this selection acts in alpine beetle communities by using tethered beetles exposed to natural predators. *Oreina gloriosa* leaf beetles (Coleoptera: Chrysomelidae) possess chemical defense in the form of cardenolides, accompanied by what appears to be warning color in bright metallic blues and greens. Individuals that match the locally predominant color morph have increased survival, with odds of week-long survival increased by a factor of 1.67 over those that do not match. This corresponds to selection of 13% against foreign morphs. Such selection, acting in concert with variation in community composition, could be responsible for geographic variation in warning color. However, in the face of this purifying selection, the within-population polymorphism seen in many *Oreina* species remains paradoxical.

KEY WORDS: **Aposematism, chemical defense, Chrysomelidae, Müllerian mimicry, natural selection, purifying selection.**

Müller's theory of warning color and mimicry is based on the following argument: in two unpalatable species that share a habitat, if they are dissimilar, predators must eat a certain number of each to learn that they are distasteful, whereas if they are identical, members of both species benefit by sharing the cost of predator education (Müller 1879; Joron and Mallet 1998; Ruxton et al. 2004). This generates selection for resemblance between unpalatable species within a community (Müllerian mimicry) and positive frequency-dependent selection (purifying selection) within a species, because in both cases common forms benefit from protection whereas rare variants suffer increased per capita predation. The principle is widely used as a textbook example of frequency-dependent selection and has considerable theoretical and comparative support (reviewed in Ruxton et al. 2004; Sherratt 2008), with renewed interest in the literature including suggestions that mimetic shifts may influence both speciation and community structure (Jiggins et al. 2001; Naisbit et al. 2003; Elias et al. 2008; Chamberlain et al. 2009). Despite this, neotropical butterflies provide the only examples for which the survival advantage enjoyed by common forms has been demonstrated using natural prey and predators in the wild (Benson 1972; Mallet and Barton 1989; Kapan 2001). This is probably in large part due to the difficulty of measuring selection in mobile species, because the confounding effect of dispersal complicates the practical and statistical techniques needed to estimate survival from recapture probabilities.

[2]These authors contributed equally to this work.

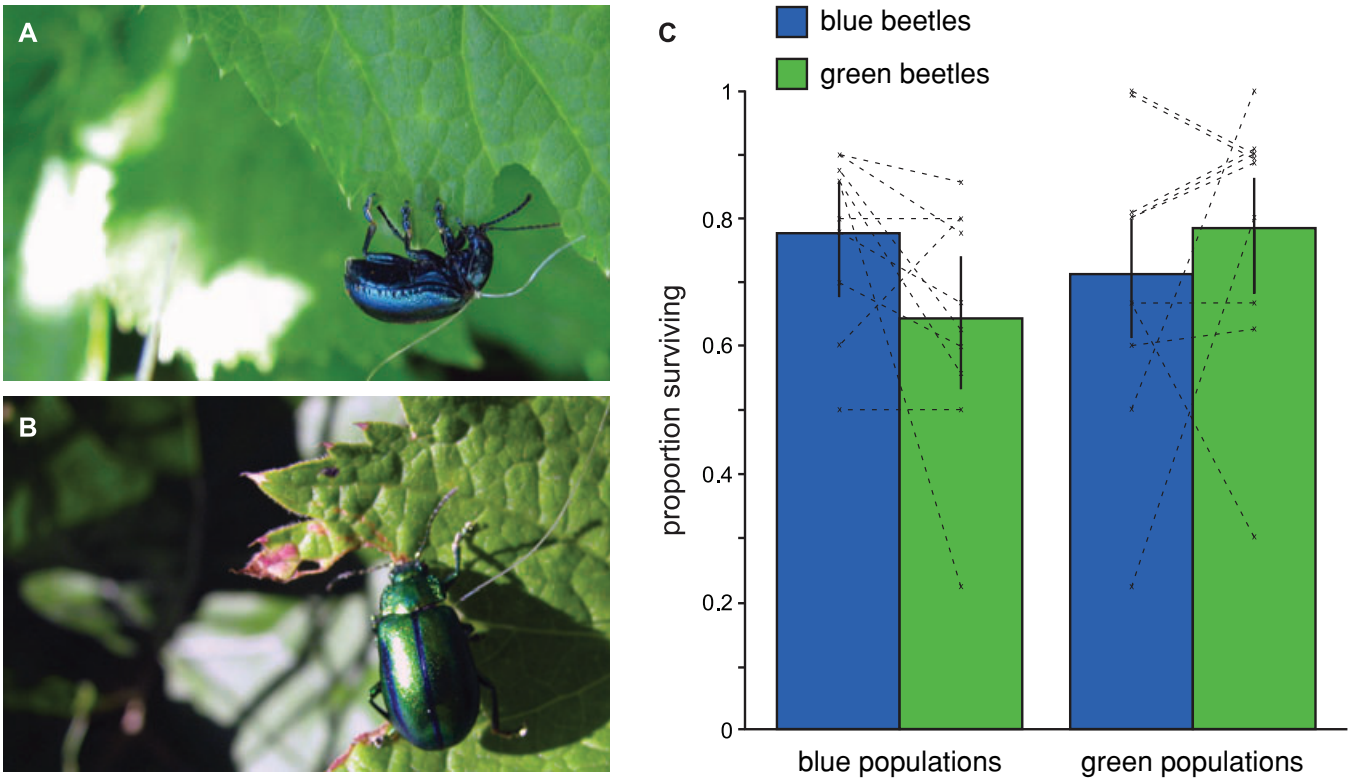

Figure 1. **(A) Blue and (B) green *Oreina gloriosa* beetles tethered on fine plastic leashes. Photographs by Tom van Noort. (C) Week-long survival estimates for blue and green beetles at blue- and green-dominated sites (10 replicates for each bar). Error bars show exact binomial (Clopper–Pearson) 95% confidence intervals for survival probability. Crosses and dashed lines indicate the results for each individual site.**

Here we avoid these problems by measuring the survival of tethered individuals in natural populations, to test for positive frequency-dependent selection on color in the alpine leaf beetle, *Oreina gloriosa* (Fig. 1). Beetles of the genus *Oreina* (Coleoptera: Chrysomelidae) are found in isolated populations throughout the mountains of Europe (Kippenberg 1994; Margraf et al. 2007; Borer et al. 2010). They possess two chemical defense strategies correlated with their host plant use. Species feeding on Apiaceae or Cynareae (Asteraceae) synthesize cardenolides, whereas those feeding on Senecioneae (Asteraceae) are able to sequester plant-derived pyrrolizidine alkaloids (Dobler et al. 1996; Triponez et al. 2007; Verdon et al. 2007). This chemical defense is accompanied by what appears to be warning coloration in bright metallic blues and greens, often in combination with blue or red stripes. Potential predators of adults include many that hunt visually, in particular birds such as the European Robin *Erithacus rubecula*, Winter Wren *Troglodytes troglodytes* and Dunnock *Prunella modularis* (van Noort personal observation), as well as predators that are less reliant on vision, like shrews, ants, and spiders.

Oreina beetles are relatively sedentary and feed exposed on the upper leaves of their host plants, so tethering them on fine plastic leashes does not greatly interfere with their natural behavior. It does, however, allow us to be certain in the identification of predation events, thereby avoiding the need to simultaneously model the unknown probabilities of resighting, dispersal, and predation from mark-recapture data (Mallet and Barton 1989; Kapan 2001). Our focus is on *Oreina gloriosa,* a species that is chemically defended by cardenolides and is monophagous on *Peucedanum ostruthium* (Apiaceae). It occurs throughout the Alps and varies in color from blue to green, in combination with three longitudinal blue stripes on the elytra. Beetles also reflect in the UV region of the spectrum, with around 25% stronger reflectance in blue compared to green morphs. We test for frequency-dependent selection on color by comparing the survival of blue and green individuals from mixed populations when exposed to predation in sites dominated by either blue or green beetles.

Methods

Experiments were carried out in communities close to the tree line (altitudes of 1592–2182 m above sea level) along the side valleys of the Rhone valley in southwestern Switzerland. In these sites the beetles are found in forest understory and open habitats in patches of a high-forb plant community that is often dominated by *Oreina* host plants, including *Peucedanum ostruthium*,

Adenostyles alliariae, *Chaerophylum villarsii*, and *Heracleum sphondylium*. Beetle color variation does not appear to be associated with habitat type.

Tethered experimental beetles were placed in 20 natural *Oreina* communities during the alpine summers between July and early September in 2005–2008. In each replicate, 10 green and 10 blue *Oreina gloriosa* beetles (always all collected from the same mixed population, either at La Fouly or Col des Mosses) were attached individually to randomly chosen *Peucedanum* plants throughout a host-plant patch. The plants were marked with plastic tags and the beetles attached using 0.4 m-long leashes made of fine transparent plastic thread (0.1 mm diameter), tied at one end around the body in the constriction between the prothorax and elytra, and at the other end to an upper node of the host plant. At the same time, the predominant color of beetles at the site was recorded, taking the entire natural *Oreina* community into account (all sites were strongly dominated by one or the other color, and included from one to six common species dominated by *O. gloriosa*, often together with *O. cacaliae* and *O. speciosa*). Sites were then visited one week later to record the survival of each tethered beetle. Preliminary trials suggested that this period would result in moderate levels of predation. On 38 out of the 400 occasions the plants could not be found again and these individuals were excluded (Supporting Information Table S1).

The association between color and survival was evaluated by using logistic regression to analyze the proportion of beetles surviving at each site. Generalized linear mixed modeling (GLMM) was used (Chapter 13 in Zuur et al. 2009), applying the lmer function in the lme4 package run within R version 2.9.2 (Bates and Maechler 2009; R Development Core Team 2009). Site name was treated as a random effect, thereby allowing us to control for differences in the overall level of predation among sites and to incorporate the correlation introduced by the paired design of the experiment. It should be noted that this term includes all factors contributing to differences in the overall level of predation among sites, including effects of year of experiment, as well as actual site-specific influences such as variation in predator communities. Beetle color was introduced as a fixed effect, coded as "local" for the beetles sharing the predominant color of the site and "foreign" for those of the other color. Two supplementary analyses were also carried out. First, beetle color was recoded as "blue" or "green," providing a test for an overall survival advantage of one morph over the other. Second, to exclude the possibility of consistent differences in the overall level of predation at blue- and green-dominated sites, the site term was instead introduced as a fixed effect with two levels ("blue-dominated" or "green-dominated"). For all these analyses, the data were well approximated by a binomial distribution (with dispersion factor of 1.19, compared to a value of 1 for an ideal binomial distribution) so there was no need to use quasibinomial estimation. The effect of beetle color was evaluated using a likelihood ratio test: when comparing models with the term included and excluded, double the difference in likelihood ($2\Delta L$) asymptotically follows a Chi-square distribution, with the degrees of freedom given by the difference in the number of parameters (one in this case).

Results

The experiment was carried out at twenty sites, half blue-dominated and half green-dominated, with overall survival values varying between 43% and 95% (Fig. 1). Beetle color had a significant effect on survival probability ($2\Delta L = 4.385$, $df = 1$, $P = 0.036$). Matching the locally predominant color increased the odds of week-long survival by a factor of 1.67 (with 95% confidence interval of 1.03 to 2.71). This advantage was similar for blue beetles in blue-dominated sites (odds ratio 1.93) and green beetles in green-dominated sites (odds ratio 1.44). There was no significant difference between blue- and green-dominated sites in their overall level of predation ($2\Delta L = 0.613$, $df = 1$, $P = 0.434$), and no overall difference in survival between blue and green beetles ($2\Delta L = 0.493$, $df = 1$, $P = 0.483$).

Discussion

Coloration had a strong effect on survival, with a significant benefit to matching the locally predominant color. Learned avoidance by visually hunting predators is therefore an important factor in the survival of *Oreina* beetles. Because our experimental sites were dominated by *Oreina gloriosa*, this represents an example of positive frequency-dependent selection on color, but there is also likely to be a contribution from Müllerian mimicry across the entire *Oreina* community.

There was variation among sites in the overall levels of predation, but this is not unexpected and would be influenced by many factors, including the weather during the replicate and the local predator density. There was also variation in the relative predation on local and foreign colors. This might be a result of natural differences in the relative frequency of blue and green morphs at each site leading to variation in the strength of frequency-dependent selection, as well as differences in the contribution of visual and non-visual predators.

The survival probabilities in our experiment can be used to estimate selection on color, because life expectancy is proportional to these values if it is assumed that the majority of predation will occur in the initial period following introduction while predators are learning to avoid the foreign patterns (Mallet and Barton 1989). The overall survival probabilities (P) of 67.7% for foreign morphs and 77.8% for local morphs translate into a selection coefficient (s) of 0.13 (using $s = 1 - P_{\text{foreign}}/P_{\text{local}}$).

This strong selection is comparable with estimates from other Müllerian mimicry systems: 0.22 in *Heliconius erato* painted to produce novel morphs (Benson 1972), 0.52 in *H. erato* transferred across a hybrid zone (Mallet and Barton 1989), and 0.64 in polymorphic *Heliconius cydno* (Kapan 2001). In our experiment, the use of leashes allows us to be certain that death due to predation is the cause of the disappearance of individuals, and because all beetles within each replicate were taken from the same source population and varied only in color, we can be sure that differential predation according to color must underlie the selection. Our value is somewhat lower than earlier estimates, possibly because we can exclude the potential confounding effects of differential dispersal or resighting. However, variation in the estimates of s could also be a result of many ecological differences between the systems, such as differences in predator communities, prey memorability or predator learning ability, the strength of deterrence due to defenses, or the background level of loss to nonvisual predators.

This mode of positive frequency-dependent selection by predators would be expected to have opposite effects on the levels of inter- and intra-population polymorphism, acting to generate geographic variation and eliminate within-population polymorphism. It forms a mechanism by which geographic variation in community composition would alter the target of convergence, and could thereby generate geographic variation in color within species (Mallet and Turner 1998). *Oreina* communities are susceptible to vary geographically in the species present because of variation in the host plants available, and as a result of stochastic effects of their limited dispersal ability, the isolated nature of their habitats, and the fact that the entire region was subjected to repeated extinction and recolonization during Quaternary glacial cycles (Hewitt 2004; Margraf et al. 2007). Local adaptation of populations towards the fitness peak formed by the predominant color of the community could therefore be responsible for the observation that several species show great variation in coloration across their distribution (Kippenberg 1994; Knoll and Rowell-Rahier 1998). In contrast, this form of frequency-dependent predation should eliminate within-population polymorphism because it generates strong purifying selection. The remarkable variation in color seen within populations of many *Oreina* species is therefore paradoxical (Joron and Mallet 1998; Mallet and Joron 1999). Future work will be devoted to examining other factors, such as variation in unpalatability (Speed 1993), neophobia (Franks and Oxford 2009), sexual selection, gene flow, or even the shape of frequency-dependent selection itself (Mallet and Joron 1999), that may be responsible for maintaining this diversity.

ACKNOWLEDGMENTS

The work was funded by Swiss National Science Foundation grants 3100–064864.01 and 3100-AO-118031 (including a doctoral studentship to TvN) and a university doctoral studentship to MB. REN is grateful to Rudolf Rohr for discussion of GLMM and we thank Tom Sherratt and one anonymous reviewer for comments on the manuscript.

LITERATURE CITED

Bates, D., and M. Maechler. 2009. lme4: linear mixed-effects models using S4 classes. R package version 0.999375–31.

Benson, W. W. 1972. Natural selection for Müllerian mimicry in *Heliconius erato* in Costa Rica. Science 176:936–939.

Borer, M., N. Alvarez, S. Buerki, N. Margraf, M. Rahier, and R. E. Naisbit. 2010. The phylogeography of an alpine leaf beetle: divergence within *Oreina elongata* spans several ice ages. Mol. Phylogen. Evol. doi: 10.1016/j.ympev.2010.08.017 [Epub ahead of print].

Chamberlain, N. L., R. I. Hill, D. D. Kapan, L. E. Gilbert, and M. R. Kronforst. 2009. Polymorphic butterfly reveals the missing link in ecological speciation. Science 326:847–850.

Dobler, S., P. Mardulyn, J. M. Pasteels, and M. Rowell-Rahier. 1996. Host-plant switches and the evolution of chemical defense and life history in the leaf beetle genus *Oreina*. Evolution 50:2373–2386.

Elias, M., Z. Gompert, C. Jiggins, and K. Willmott. 2008. Mutualistic interactions drive ecological niche convergence in a diverse butterfly community. PLoS Biology 6:2642–2649.

Franks, D. W., and G. S. Oxford. 2009. The evolution of exuberant visible polymorphisms. Evolution 63:2697–2706.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Phil. Trans. R. Soc. Lond. B 359:183–195.

Jiggins, C. D., R. E. Naisbit, R. L. Coe, and J. Mallet. 2001. Reproductive isolation caused by colour pattern mimicry. Nature 411:302–305.

Joron, M., and J. L. B. Mallet. 1998. Diversity in mimicry: paradox or paradigm? Trends Ecol. Evol. 13:461–466.

Kapan, D. D. 2001. Three-butterfly system provides a field test of Müllerian mimicry. Nature 409:338–340.

Kippenberg, H. 1994. Familie Chrysomelidae. Pp. 65–83 *in* G. A. Lohse and W. H. Lucht, eds. Die Käfer Mitteleuropas, 3 Supplementband. Goecke & Evers, Krefeld, Germany.

Knoll, S., and M. Rowell-Rahier. 1998. Distribution of genetic variance and isolation by distance in two leaf beetle species: *Oreina cacaliae* and *Oreina speciosissima*. Heredity 81:412–421.

Mallet, J., and N. H. Barton. 1989. Strong natural selection in a warning-color hybrid zone. Evolution 43:421–431.

Mallet, J., and M. Joron. 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Annu. Rev. Ecol. Syst. 30:201–233.

Mallet, J. L. B., and J. R. G. Turner. 1998. Biotic drift or the shifting balance – did forest islands drive the diversity of warningly coloured butterflies? Pp. 262–280 *in* P. R. Grant and B. Clarke, eds. Evolution on islands. Oxford University Press, Oxford.

Margraf, N., A. Verdon, M. Rahier, and R. E. Naisbit. 2007. Glacial survival and local adaptation in an alpine leaf beetle. Mol. Ecol. 16:2333–2343.

Müller, F. 1879. *Ituna* and *Thyridia*; a remarkable case of mimicry in butterflies. Trans. Entomol. Soc. Lond. 1879:xx–xxix.

Naisbit, R. E., C. D. Jiggins, and J. Mallet. 2003. Mimicry: developmental genes that contribute to speciation. Evol. Dev. 5:269–280.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Ruxton, G. D., T. N. Sherratt, and M. P. Speed. 2004. Avoiding attack. Oxford University Press, Oxford.

Sherratt, T. N. 2008. The evolution of Müllerian mimicry. Naturwissenschaften 95:681–695.

Speed, M. P. 1993. Muellerian mimicry and the psychology of predation. Ani. Behav. 45:571–580.

Triponez, Y., R. E. Naisbit, J. B. Jean-Denis, M. Rahier, and N. Alvarez. 2007. Genetic and environmental sources of variation in the autogenous chemical defense of a leaf beetle. J. Chem. Ecol. 33:2011–2027.

Verdon, A., N. Margraf, A. C. Davison, M. Rahier, and R. E. Naisbit. 2007. Conserved oviposition preferences in alpine leaf beetle populations despite host shifts and isolation. Ecol. Entomol. 32:62–69.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York.

Associate Editor: C. Jiggins

Supporting Information

The following supporting information is available for this article:

Table S1. Numbers of blue and green beetles surviving and suffering predation at each site.

Supporting Information may be found in the online version of this article.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.