

Density-Dependent Melanism in Winter Moth Larvae (Lepidoptera: Geometridae): A Countermeasure Against Parasitoids?

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ABSTRACT Density-dependent melanism, i.e., the phenomenon that individuals at high population densities develop into a dark-colored phenotype, is often explained as a defense against density-dependent responses of natural enemies, in particular, disease organisms. In the work reported here, we wanted to test whether density-dependent melanism in insects may yield protection against parasitoids, which are important regulators of many outbreak populations. This was carried out by collecting comprehensive field data on parasitoid attack rates and overall mortality of both larvae and pupae of the outbreak species *Operophtera brumata* L. (winter moth) in relation to degree of cuticular melanism. As expected, the results showed that parasitoids were the dominating mortality factor, but unexpectedly, parasitoid attack was positively associated with the degree of melanism. Also, mortality caused by unknown factors seemed to be highest in melanic larvae. These results indicate that density-dependent melanism, which is known to protect several species of insects against disease agents, does not confer protection against parasitoids in this outbreak species, which is largely regulated by parasitoids in nature.

KEY WORDS *Operophtera brumata*, melanism, density-dependent, parasitoid

Many outbreak species of insects develop into a dark-colored phenotype at high population densities (Long 1953, Kunimi and Yamada 1990, Goulson and Cory 1995, Reeson et al. 1998, 2000, Barnes and Siva-Jothy 2000, Hagen et al. 2003). Such density-dependent or crowding-induced melanism is often explained as a direct countermeasure against density-dependent responses of natural enemies (predators, parasites, disease) (Kunimi and Yamada 1990, Reeson et al. 1998, Barnes and Siva-Jothy 2000, Wilson et al. 2001). This is because melanin apparently is toxic to microbes (Ourth and Renis 1993), because melanin indirectly seems to be associated with immune defense directed against pathogens and parasites (Poinar 1974, Götz 1986, Hung and Boucias 1992, Beckage et al. 1993, Cotter et al. 2004), and because melanin is believed to reduce surface penetration of disease organisms by strengthening the insect's cuticle (St. Leger et al. 1988, Hajek and St. Leger 1994, Wilson et al. 2001). However, as far as we know, no attempt has yet been made to estimate attack rates by natural enemies on melanic and nonmelanic phenotypes in an actual field situation. Thus, at this time, the role of density-dependent melanism in regulating enemy attack in natural insect populations remains uncertain.

The aim of this study was to provide a first test of the relationship between cuticular melanism and enemy

attack rate in larvae of an outbreak species its natural environment. This was done by estimating attack rates by parasitoids and overall mortality of larvae in relation to degree of cuticular melanism in a natural population of the geometrid moth species *Operophtera brumata* L. (winter moth) in northern Norway. The winter moth shows cyclic population dynamics, with outbreaks occurring approximately every 9–10 yr in birch forests (*Betula pubescens* Ehrh.) of this region (Tenow 1972, Hogstad 1997, Neuvonen et al. 1999). The larvae of the species are highly variable in coloration, from pale yellow or green to almost entirely black, and exhibit direct density-dependent melanism (Hogstad 1996, Hagen et al. 2003). Natural enemies, in particular insect parasitoids, have often been proposed as a likely causal mechanism underlying the cyclic population dynamics of northern Geometrid populations (for recent reviews, see Ruohomäki et al. 2000, Klemola et al. 2002). Parasitoid attack is clearly the most important larval mortality factor displaying density dependence in the winter moth (Roland and Embree 1995) and in the related, sympatric autumnal moth (*Epirrita autumnata* Bkh. [Lepidoptera: Geometridae]) (Ruohomäki et al. 2000, Klemola et al. 2002). Thus, assuming that density-dependent melanism(s) underlying this species' cyclic population dynamics, detailed data on the link between melanism and parasitoid attack in natural populations may help direct future research on the causes of these cycles.

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Table 1. Sample sizes and proportions, with 95% confidence levels (in brackets), of dead larvae (unknown causes), dead pupae (unknown causes), and larvae parasitized at two altitudes

Variable/category	170 m	240 m
Proportions dead larvae		
Melanic	0.120 ($n = 195$) [0.080, 0.170]	0.140 ($n = 141$) [0.094, 0.210]
Intermediate	0.039 ($n = 254$) [0.022, 0.071]	0.039 ($n = 229$) [0.021, 0.073]
Nonmelanic	0.031 ($n = 257$) [0.016, 0.060]	0.099 ($n = 424$) [0.074, 0.130]
Proportions dead pupae		
Melanic	0.023 ($n = 172$) [0.009, 0.058]	0.050 ($n = 121$) [0.023, 0.100]
Intermediate	0.041 ($n = 244$) [0.022, 0.074]	0.077 ($n = 220$) [0.049, 0.120]
Nonmelanic	0.032 ($n = 249$) [0.016, 0.062]	0.079 ($n = 382$) [0.056, 0.110]
Proportions parasitised larvae		
Melanic	0.43 ($n = 168$) [0.36, 0.50]	0.36 ($n = 115$) [0.27, 0.45]
Intermediate	0.34 ($n = 235$) [0.28, 0.40]	0.25 ($n = 203$) [0.19, 0.31]
Nonmelanic	0.38 ($n = 241$) [0.32, 0.44]	0.14 ($n = 352$) [0.11, 0.18]

Materials and Methods

Study Area. Field work was carried out during June and July 2003 in a coastal, subarctic birch forest located at the island of Reinøya in Troms county, northern Norway (70°00' N, 19°49' E). The study area was located in a northeasterly oriented slope (mean slope, 23.3°) where the forest forms a fairly homogenous belt from sea level and up to a rather sharp forest limit at ≈240–250 m elevation. The birch forest at the study site is a mosaic of the heath and the meadow type (Hämet-Ahti 1963), with scattered occurrence of rowan, *Sorbus aucuparia* L., and willow, *Salix* spp. (see Mjaaseth et al. 2005 for detailed description).

Study Design. Ten parallel altitudinal transects spaced at 200-m intervals were established. Each transect had four sampling stations at 30, 100, 170, and 240 m elevation, respectively, where winter moth larvae were sampled to determine the role of cuticular melanism in regulating parasitoid attack as well as overall mortality of larvae and pupae. Only samples from 170 and 240 m will be considered in this study, because of the low number of melanic larvae obtained from the two lower altitudes, where population densities were low.

As an index of local population density, larvae were sampled from 10 arm-length birch twigs, collected haphazardly from different trees, within a radius of 20 m of each sampling station (Hagen et al. 2003, Ins et al. 2004). Each twig was thoroughly beaten with a stick over a large plastic box, and the number of larvae was counted. The 20 sampling stations included in this study spanned a fairly large range of local densities (from 0.1 to 6.3 larvae per twig). A larger number of larvae for analysis of mortality factors under specific rearing conditions were obtained from each sampling station using the same sampling method.

The larvae, which were approximately in their fourth instar at the time of sampling, were subjectively classified into three ordinal categories based on their degree of cuticular melanism (Hagen et al. 2003): (1) nonmelanic (pale yellow or green larvae with pale head capsules), (2) melanic (very dark larvae with black stripes and black head capsules), or (3) intermediate (larvae being between these two extremes of coloration).

Larvae of each morph from the 20 sampling stations (i.e., from 170 and 240 m elevation) were taken to the laboratory and kept separately at room temperature (≈20°C) in groups of 10. Each larval group was kept in a covered 1-liter plastic box (8 by 15 by 8 cm) containing an ≈1-cm-thick layer of silted sand in which larvae could pupate. Sphagnum moss was added to retain moisture. A food supply of fresh birch leaves was added to each box every other day until all of the caterpillars had either died or pupated. Emergence of parasitoids, as well as deaths caused by other sources (unidentified), was recorded concurrently with feeding.

When all larvae had either died or pupated, the boxes were moved outside and kept at ambient temperatures, sheltered from sun and precipitation. The boxes were checked every 3 d from 26 September to 12 October, and the number of adults hatching from each box was recorded. Pupae/cocoons that did not hatch and had no parasitoid inside were recorded as having died from an unknown cause. Table 1 shows the total material obtained in this study.

Statistical Procedure. Sources of variation in the mortality between the 20 sampling stations were studied using logistic regression analysis (Agresti 1990). The three focal response variables, which were analyzed separately, were the proportion of larvae found dead, of pupae found dead (after removing the larvae known to be dead), and larvae being parasitized (of those known to have survived up to the pupal stage). The former two proportions correspond to unknown causes of death. In a fourth and final analysis, we also examined the timing of hatching, as the proportion that hatched before 26 September, to explore whether melanic and nonmelanic larvae differed in hatching phenology. In all of the analyses, the focal predictor variable was cuticular melanism with the tree nominal levels melanic, intermediate, and nonmelanic. We also included two covariates that we suspected might have influenced the mortality patterns. These were local (i.e., station specific) population density (as a quantitative variable) and sampling altitude (categorical with two levels). Altitude was analyzed as categorical instead of continuous because there were data from only two altitudes and because this approach gave the

Table 2. Results from model selection based on AIC of sources of variation in mortality of larvae and pupae and in eclosion time of adults

A	C	D	AC	AD	CD	ACD	Dead larvae	Dead pupae	Parasitism	Eclosion time
x	x	x	x	x	x	x	193.6	159.9	137.7	115.2
x	x	x	x	x	x		193.6	155.0	136.1	115.2
x	x	x	x	x			189.5	149.0	131.7	109.7
x	x	x	x		x		191.0	159.6	136.7	116.2
x	x	x		x	x		189.5	149.0	136.8	111.7
x	x	x	x				187.8	153.8	132.5	108.9
x	x	x			x		187.1	153.9	136.9	112.6
x	x	x		x			189.1	143.5	132.6	109.2
x	x		x				187.8	157.8	130.0	104.9
x	x	x			x		184.9	151.9	142.0	108.9
x		x		x			207.6	140.4	134.7	102.6
x	x	x					188.1	148.7	132.6	107.3
x	x	x					186.5	146.9	138.2	104.5
x		x					206.7	145.8	134.2	101.3
x	x						188.6	153.0	130.3	104.6
x							207.4	150.5	132.2	99.2
	x						193.0	160.9	143.9	101.7
		x					204.9	144.4	141.6	99.1
							210.6	159.7	149.1	96.8
							AICc	AICc	QAICc	AICc

For each of the four proportions or response variables, the two best models (i.e., those with the lowest AIC values) are highlighted in bold. From any one of the highlighted AIC values, follow the row to the left to see the predictor variables contained in the selected model. The predictor variables considered were A = altitude, C = color (i.e., degree of melanism), and D = larval density, plus all possible interactions among these variables (denoted by combining letters: AC = interaction between A and C).

best model fit as judged from various diagnostic plots. In all analyses, we used a model selection approach based on AICc (Akaike's Information Criterion corrected for small sample size: Burnham and Anderson 1998) to find the most parsimonious models describing the variation in the different proportions studied in this paper. We first assessed the goodness-of-fit of the most complex model (all main terms and interactions among melanism, density, and altitude) using residual plots and overall goodness-of-fit based on the sum of Pearson squared residuals (McCullagh and Nelder 1989). There was evidence of overdispersion for the analysis of proportion larvae parasitized ($\chi^2 = 90.79$; $df = 38$; $P < 0.01$), and we therefore used QAICc (AICc corrected for overdispersion) for this proportion with an inflation factor $c = 2.54$ (Burnham and Anderson 1998). Note that when the inflation factor $c = 1$, the formulae for QAIC and QAICc reduce to AIC and AICc. There was otherwise no evidence for overdispersion for the other proportions (dead larvae: $\chi^2 = 52.34$; $df = 38$; $P > 0.05$; dead pupae: $\chi^2 = 44.27$; $df = 38$; $P > 0.10$; timing of hatching: $\chi^2 = 13.69$; $df = 15$; $P > 0.10$). Residual plots showed that there were some influential observations, but because the estimates and main conclusions were not affected by their exclusion, we chose to include them when presenting the results. Confidence intervals for proportions were calculated according to the Wilson method (Agresti and Coull 1998). All analyses were carried out using the statistical software R (R Development Core Team 2004).

Results

Overall, the highest mortality rate was accounted for by parasitoids (26% of the total number of larvae;

Table 1). The overall mortality rate due to unknown causes was similar in larvae (7%) and in pupae (5%).

Logistic modeling of the different sources of mortality at the level of sampling stations showed that both mortality caused by parasitoids and unknown mortality in larvae were related to the degree of melanism (Table 2). However, in contrast to the hypothesis' prediction that melanism is a counteraction against enemies, the probability for larvae being attacked by parasitoids increased with the degree of melanism (Table 1). The best logistic model indicated that effect of melanism on parasitism interacted with altitude so that nonmelanic larvae were least attacked at the highest altitude (Table 1). However, the model with an interaction was only marginally better than a model with degree of melanism and altitude as additive effects (Table 2). Also, mortality from unknown sources in larvae was consistently higher in melanic larvae (Table 3), although the best logistic model indicates that this difference may depend on local population density (Tables 2 and 3). Neither unidentified mortality agents in pupae or timing of eclosion of adults from pupae showed any relationship with degree of larval melanism.

Discussion

This study found no evidence that crowding-induced melanism in winter moth larvae is a counteraction against density-dependent attacks of natural enemies. On the contrary, parasitoid attack, which is clearly the most important larval mortality factor exhibiting density-dependence in the winter moth (Roland and Embree 1995) and the related sympatric autumnal moth in subarctic birch forest (Ruohomäki et al. 2000, Klemola et al. 2002), seemed to be highest

Table 3. Proportion of dead larvae (with 95% confidence intervals in brackets) caused by unknown sources according to degree of melanism and local population density

Category	Density: 0.1–2.1 larvae/twig	Density: 2.2–4.2 larvae/twig	Density: 4.3–6.3 larvae/twig
Melanic	0.14 ($n = 207$) [0.10, 0.20]	0.11 ($n = 198$) [0.070, 0.16]	0.15 ($n = 40$) [0.071, 0.29]
Intermediate	0.041 ($n = 242$) [0.023, 0.074]	0.044 ($n = 181$) [0.023, 0.085]	0.017 ($n = 60$) [0.001, 0.089]
Nonmelanic	0.098 ($n = 419$) [0.073, 0.130]	0.045 ($n = 202$) [0.023, 0.082]	0.00 ($n = 60$) [0, 0.060]

For the purpose of obtaining estimated proportions with confidence intervals, local population densities (i.e., average no. of larvae per twig per sampling station) were categorized according to three intervals.

in melanic larvae. Even the relatively less common unidentified mortalities among larvae in our study, which presumably included incidents of pathogens such as virus and fungi, were most frequent among melanic larvae.

This study is to our knowledge the first that exploits observational field data to address the question whether mortality inflicted by natural enemies is color morph dependent in a cyclically fluctuating Geometrid moth population. Although our data do not indicate any adaptive advantage of melanism, it is still hard to imagine that melanism is induced by crowding for no particular reason. Therefore, further observational and experimental studies are warranted. Further studies on color morph-dependent mortality caused by natural enemies should include the extreme densities that may be encountered during some population outbreaks in which some disease organisms like virus and fungi may play a greater role. Moreover, during extreme outbreak densities melanism may serve other condition-dependent functions. For instance, during large outbreaks the foliage of the host trees typically becomes much sparser and darker (unpublished data). Against this background, the dark coloration of melanic larvae may serve as a better camouflage than a pale green coloration. The sparser foliage at outbreak densities may also expose the larvae to more UV radiation against which pigmentation offers protection (Gunn 1998). There is also evidence that melanism may play a role in temperature regulation, with darker phenotypes absorbing more sun energy, yielding faster growth (Goulson 1994, Hazel 2002) that could compensate for resource limitations during outbreaks. That the function of melanism may exhibit several, yet unexplored forms of condition dependence, is also indicated by our finding that the relation between melanism and larval mortality interacted with local population density and altitude.

In conclusion, the adaptive significance of direct density-dependent melanism in winter moth larvae remains uncertain; however, this study does not support the possibility that this phenomenon works as an effective defense action against parasitoids, the most important enemies during the larval stage in this species.

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