Effects of polymorphic melanism and larval diet on life history traits of *Malacosoma disstria* moths

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Abstract

In this study we investigated the presence and possible genetic basis of polymorphic melanism in the forest tent caterpillar (*Malacosoma disstria*) moth. Adult moths were classified into pattern-based phenotypes and wing darkness was measured to quantify the degree of melanization. We found that two distinct phenotypes, melanic and simple, are present in these moths. Although the full melanic phenotype is sex-limited to males, it is partially expressed in females. We also provide support for the theory that the melanic allele is autosomal and dominant. The effects of larval diet quality on the survival, development and wing melanization of each phenotype were studied by rearing larvae on the foliage of either a primary or secondary host. Diet quality did not differentially affect the two phenotypes; however, melanic males were found to be smaller than simple males regardless of larval diet. Such inherent developmental differences between the two phenotypes could have important consequences for the frequencies of the two morphs.

Keywords

Melanism; colour polymorphism; wing pattern; Lepidoptera; development; forest tent caterpillar

1. Introduction

Many Lepidoptera have wing colour polymorphisms where two or more distinct phenotypes derive from multiple alleles at a single gene locus (or a few tightly linked gene loci) (Beldade and Brakefield 2002). In moths, melanins are the most common colour pigments, giving rise to various shades of colour from white or pale brown to dark brown or black. Individuals that have synthesized and deposited more melanin in their cuticle have darker phenotypes and are termed melanic (Kettlewell 1973; Majerus 1998). Basic crossing experiments have indicated that adult moth melanic polymorphisms are often controlled by a single gene locus, with melanic alleles being dominant (e.g. Lees 1974; Lorimer 1979; West 1977; also see Beldade and Brakefield 2002; Cook *et al.* 2002; Kettlewell 1973). More rarely, polymorphic melanism is inherited as a Mendelian recessive in either the adult or the larvae, or over multiple stages of development (Bear *et al.* 2010; Futahashi *et al.* 2008; Kettlewell 1973; Majerus 1981).

A well-known case of an adult dominant melanic polymorphism is that of the peppered moth, *Biston betularia*, because of drastic increases in the frequency of melanic phenotypes in certain areas of Great Britain as a result of the industrial revolution (e.g. Berry 1990; Cook 2003; Grant 1999; Kettlewell 1973; Majerus 1998). However, melanic phenotypes are also present at low frequencies in rural populations of *B. betularia* unaffected by industrial emissions (Cook *et al.* 2002). Melanic phenotypes have also been documented in populations of several other moth species in Great Britain (Cook *et al.* 2002; Lees 1971) and North America (Lorimer 1979; Owen 1962; Sargent 1985; West 1977). Unfortunately, the ecological and physiological impacts of melanic phenotypes have received little attention outside the context of industrial melanism, and the selection pressures maintaining polymorphisms in these populations are unknown.

Melanin pigments are not ingested; rather, they are synthesized from amino acid precursors and are rich in nitrogen (e.g. Blarzino *et al.* 1999; McKinnon and Pierotti 2010; Nijhout 1985; Stoehr 2006). Nitrogen is already an important, and often limiting, nutrient for herbivorous insects (e.g. Schowalter 1986; White 1984); and indeed, high levels of melanization have been shown to be costly in some insects. In the leaf beetle, *Chrysomela lapponica*, dark morph beetles had much higher hibernation mortality than pale morph beetles after the beetles were fed on a low quality diet (Zvereva *et al.* 2002). Talloen *et al.* (2004) found that darker-winged *Pararge aegeria* butterflies had longer larval stages and more asymmetrical wings, independent of diet quality. However, the cost of melanin production may be offset by a decrease in melanin synthesis when conditions are poor. Wing melanization of *Pararge aegeria* butterflies was reduced when the larvae were reared on a low quality diet (Talloen *et al.* 2004). Similarly, Allegret (1964) reported that protein deprivation in the late larval stages of *Galleria mellonella* resulted in moths with reduced pigmentation.

An interesting model species with which to investigate polymorphic melanism and the effects of larval diet quality on the development, survival, and melanization of individuals of different phenotypes is the forest tent caterpillar, *Malacosoma disstria*. *M. disstria* is a North American pest insect with outbreak population dynamics and a large distribution that covers the United States and southern Canada from coast to coast (Fitzgerald 1995). High levels of intra-population variation in wing colour are present in *M. disstria* moths, particularly in males (Stehr and Cook 1968). Male wings not only range in colour from very dark brown to pale tan, but also exhibit differences in wing patterns, whereas female wing colour is more homogenous in both colour and pattern (Lorimer 1979).

Lorimer proposed that melanism in *M. disstria* is polymorphic but sex-limited to males, with autosomal dominant inheritance. She tested possible mechanisms for inheritance of the melanic allele by comparing the observed frequency of family types (i.e. all males melanic, all males non-melanic, or a mix of both) to those expected if the melanic allele is autosomal dominant, autosomal recessive, sex-linked dominant, or sex-linked recessive, and found that the autosomal dominant mode of inheritance best fit the data. Furthermore, she found that the observed phenotypic ratios of males within mixed families most closely resembled those expected if the melanic allele is autosomal and dominant. Finally, Lorimer found no evidence of the melanic allele being lethal in females; this suggests that females carry the melanic allele but simply do not express the phenotype.

M. disstria moths are non-feeding; all the energy and nutrients required for an individual to successfully metamorphose into an adult and survive to fulfill its reproductive functions are accumulated during the larval stage (Fitzgerald 1995). Environmental conditions experienced by the larvae can therefore be expected to have a direct effect on the physical traits of the adult moth through irreversible allocation of available resources during the pupal stage. Furthermore, the high insect population densities that occur during outbreaks result in a decrease in both the quantity and quality of food available to individual larvae; hence, they suffer periodic starvation and are often forced to feed on secondary host trees (Fitzgerald 1995). Due to the cost of melanin production, melanic and non-melanic phenotypes may be expected to show differences in life history traits, such as development time and body size. Melanic males may also suffer increased mortality, particularly under suboptimal conditions such as those experienced during outbreaks. Alternatively, the negative effects of increased melanization could be counteracted by an induced reduction in melanization under poor conditions.

In this study we test several hypotheses. The insects used in Lorimer's (1979) experiment were collected from Michigan, Minnesota, Indiana, and Alabama (USA). However, as mentioned previously, this species' distribution spans a large part of North America. Lorimer's hypothesis of a sex-limited, autosomal dominant melanic allele is therefore further tested here on a sample collected from a population in northern Alberta (Canada), much further north and west than any of Lorimer's sample populations. Comparing phenotypic ratios within and between families to those expected by autosomal dominance of the melanic allele (as per Lorimer 1979) will determine whether the polymorphism, and its proposed genetic basis, is widespread in *M. disstria*. Furthermore, Lorimer's (1979) melanic phenotype, which she termed 'Dark', was based on a purely visual assessment of wing darkness and patterns. Here, moths will similarly be classified into phenotypes, but on the basis of wing patterns only. Wing darkness will then be quantified as an indicator of melanization and compared between pattern-based phenotypes to test the hypothesis that one phenotype is measurably darker than the other, and therefore melanic. Finally, the effects of larval diet quality on the development, survival, and melanization of moths will be investigated to determine whether differences exist between phenotypes.

2. Methodology

2.1 Larval rearing

Unhatched egg masses were collected in the early spring of 2008 from a high density (outbreak) population near Wabasca in northern Alberta, Canada (56°17.5N, 113°93.9W). They were stored at 4°C and 80%R.H. until local budbreak of trembling aspen (*Populus tremuloides*), the primary host of northern populations of *M. disstria* (e.g. Parry and Goyer 2004; Stehr and Cook 1968).

Egg masses were sterilized in a 5% bleach solution before hatching (Grisdale 1985). All larvae hatching from a single egg mass can be assumed to be full siblings (Fitzgerald 1995 and references therein) and are henceforth referred to as a family. Families (n=28) were reared in separate plastic containers at 21±1°C and 70%R.H., under a 16hr light: 8hr dark photoperiod.

Larvae were fed fresh trembling aspen leaves *ad libitum* from time of hatch to the middle of the fourth larval instar. Once all larvae within a family finished moulting to the fourth instar, the family was haphazardly divided in two and each half was moved to a new container. One half was fed trembling aspen leaves and the other half was fed white birch (*Betula papyrifera*) leaves until completion of the larval stage. White birch is a less-preferred secondary host in northern *M. disstria* populations (Hodson 1941), and represents the low-quality food that larvae may be forced to feed on during outbreaks.

In seven families, infection and mortality from the highly epizootic nuclear polyhedrosis virus was so prolific in the fourth or fifth instar that the colonies were lost and could not be included in this study. Pupae were weighed and placed in small individual containers; immediately after eclosion, the moths were stored at -16°C until processing of the wings. Development time was recorded for each individual as the number of days from hatching of the egg mass to eclosion of the moth. Individuals that failed to construct a silk cocoon and pupated on the bottom of the container were recorded as having no cocoon.

2.2 Characterizing adult wing patterns and melanization

Each moth was sexed and visually classified as having a patterned or simple phenotype based on the presence or absence of specific pattern elements on the forewing (Figure 1). Patterned moths have two pale bars on their forewings adjacent to the dark proximal and distal bars (as did

Lorimer's (1979) 'Dark' phenotype), as well as greater variation in shading along the top edge of the forewing (pers. obs.). These extra pattern elements were only observed in males, so females were all classified as simple. Thus, moths were divided into three 'adult types': patterned males, simple males, and (simple) females (Figure 1). In all three adult types, colouration of the wings reflected overall colouration of the body (pers. obs.).

The following colour scoring procedure is based on a procedure developed by Dr. Maya Evenden of the University of Alberta (pers. comm.) and is similar to procedures described in other studies on animal colouration (e.g. Davis *et al.* 2005; Eakley and Houde 2004; Holloway *et al.* 1997; Punzalan *et al.* 2008a; Robertson and Robertson 2008; Talloen *et al.* 2004). One forewing was removed from each moth and glued to a sheet of white paper; sheets of wings were then scanned using an HP Scanjet 5590 scanner (along with a paper cm/mm ruler to provide scale). Using the software ImageJ 1.40g (Rasband 1997-2011), each file was converted to an 8-bit greyscale (0 = black, 255 = white) in order to measure darkness as a proxy for melanization (e.g. Talloen *et al.* 2004).

For each moth, the area in mm^2 and the mean grey value of the forewing were recorded. Boxes measuring 15x15 pixels were used to measure mean grey values of the proximal and distal areas of the forewing (Figure 1). These values were then averaged to find the background darkness of the forewing. Due to the nature of the greyscale, a low grey value indicates darker wings and increased melanization compared to a high grey value. Background darkness was found to be a more reliable index than the mean grey value of the whole forewing, as any areas of damage (such as wrinkles or loss of colour scales) were purposely avoided during placement of the boxes used to score background darkness. Moths with extensively damaged wings were not used. Some moths failed to properly expand their wings after eclosion. Although these unexpanded wings

could not be colour scored, many could still be classified by phenotype and were therefore included when calculating phenotypic frequencies.

2.3 Statistical analyses

A paired t-test was used to determine whether the low-quality birch diet differentially affects survival of male phenotypes by comparing phenotypic frequencies within families across diet treatments. Chi square tests were performed to compare observed frequencies of male phenotypes within and between families to those expected if the patterned allele is autosomal and dominant to the simple allele (as per Lorimer 1979). For this part of the analysis only, alpha was increased to 0.10 instead of the standard 0.05 in order to increase power and reduce the risk of committing a Type II error (failing to reject a false null hypothesis). It is usually more serious to commit a Type I error (rejecting a true null hypothesis) as this will erroneously provide support for the research hypothesis. In this particular case, however, failing to reject the null hypothesis supports the theory being tested. By increasing alpha to 0.10, the risk of erroneously finding support for the proposed theory is reduced. The effects of adult type and diet on wing darkness (melanization), development time, pupal mass, and forewing area were tested using an analysis of variance (ANOVA). The ANOVA controlled for the effect of family (as a random factor) while testing for effects of adult type and diet, and included all 2- and 3-way interactions. Tukey post hoc tests were performed when necessary. All statistical analyses were performed in SPSS v.16.0 (IBM 2007), except for the Chi square tests, which were calculated using Excel (Microsoft Office 2007).

3. Results

3.1 Genetic basis of phenotypes

Families can be classified into three types based on the types of males present: all males simple, all males patterned, or a mix of both. These families will henceforth be referred to as simple families (n=9), patterned families (n=2), and mixed families (n=10). Simple and patterned families remained so regardless of diet treatment. In mixed families, ratios of patterned to simple males were first calculated separately for family halves (groups raised on either aspen or birch) and compared using a paired-samples t-test. It was found that diet treatment had no significant effect on the ratio of patterned to simple males within families (d.f.=9, t=0.673, p=0.518); thus, diet did not differentially affect survival of the two types of males. The Chi square tests for phenotypic ratios of mixed families were therefore performed on entire families (i.e. both diet treatments combined).

According to Lorimer (1979), polymorphic melanism in *M. disstria* is controlled by two alleles at a single gene locus, the patterned allele being dominant but only expressed in males. From this hypothesis, the expected ratio of patterned to simple males in mixed families is either 3:1 or 1:1, depending on the parental cross. This prediction was tested using Chi square tests (α=0.10), which showed that nine of the ten mixed families were not significantly different from one of the two ratios and the remaining family was not significantly different from either ratio (Table 1). These results support the proposed hypothesis that the patterned allele is autosomal and dominant. Furthermore, the expected frequencies of each family type were calculated from the observed numbers of patterned and simple males using Hardy-Weinberg equations (patterned: p=0.178; simple: q=0.822; expected family frequencies: simple=0.456, patterned=0.063, mixed=0.482). The results of the Chi square test showed there was no significant difference

between the observed and expected number of families in each category if the patterned allele is autosomal and dominant (d.f.=2, χ^2=0.392, p=0.822).

3.2 Wing melanization and development of adult types

Separate ANOVAs were performed for mixed and simple families, as mixed families include all three adult types (patterned males: n=153; simple males: n=133; females: n=326) while simple families contain only simple males (n=297) and females (n=309). Patterned families were not analyzed due to small sample sizes (patterned males: n=54; females: n=38). All residuals were found to be normally distributed prior to analysis. Standard deviations were reported to accurately represent variability within samples (Curran-Everett 2008).

Wing darkness was significantly affected by adult type in mixed families ($F_{2,19.180}$=130.001, p<0.001). Tukey post hoc tests showed that each type is significantly different from all other types (all p<0.001) (Figure 2i). Patterned moths (always male) were found to be significantly darker than simple moths (both male and female), and will therefore be referred to as melanic for the remainder of this paper. Although females were found to be significantly darker than simple males in mixed families, there was no significant difference in wing darkness between females and simple males in simple families ($F_{1,8.624}$=0.988, p=0.347) (Figure 2ii). This suggests that females from mixed families have darker wings than those from simple families. This was tested using a simple nested ANOVA where family (still a random factor) is nested within a fixed term representing the presence or absence of melanic brothers. All three types of families were included in this analysis, but no distinction was made between mixed and melanic families. The results of the nested ANOVA showed that females with melanic brothers (n=364) had significantly darker wings than females without melanic brothers (n=309) ($F_{1,19.330}$=10.833,

p=0.004). However, the presence of melanic brothers did not have a significant effect on the development time, pupal mass, or forewing area of females. Diet had no significant effect on wing darkness in either mixed or simple families (Figure 2).

Development time was significantly affected by adult type in both mixed families ($F_{2,20.789}$=4.775, p=0.020) and simple families ($F_{1,12.453}$=43.366, p<0.001) (Figure 3i, ii). In simple families, this simply represented the expected difference between sexes. Similarly, Tukey post hoc tests for mixed families showed that the two types of males did not differ significantly in their development times (p=0.645), but both had significantly shorter development times than females (both p<0.001). Development time was also significantly affected by diet in both mixed ($F_{1,9.533}$=61.677, p<0.001) and simple families ($F_{1,8.832}$=76.346, p<0.001) (Figure 3iii, iv).

Body size was measured at two stages of development as pupal mass and adult forewing area. In mixed families, pupal mass was significantly affected by adult type ($F_{2,19.464}$=391.495, p<0.001). Tukey post hoc tests revealed that both types of males were significantly smaller than females (both p<0.001) and melanic males weighed significantly less than simple males (p=0.013) (Figure 4i). In simple families, adult type was also found to have a significant effect on pupal mass ($F_{1,10.441}$=968.782, p<0.001), which simply represented the expected difference between sexes (Figure 4ii). Diet significantly affected pupal mass in simple families ($F_{1,8.903}$=12.417, p=0.007) but not in mixed families (Figure 4i, ii).

To test forewing area, a number of moths had to be removed from the analysis due to wing damage that artificially reduced wing area values. This resulted in a decrease in sample sizes in both mixed families (melanic males: n=147; simple males: n=127; females: n=274) and simple families (simple males: n=283; females: n=263). In mixed families, forewing area was

244 significantly affected by adult type ($F_{2,21.155}$=845.145, p<0.001). Tukey post hoc tests indicated
245 that, as was the case with pupal mass, both types of males were significantly smaller than
246 females (both p<0.001) and melanic males were significantly smaller than simple males
247 (p=0.011). Unlike pupal mass, however, forewing area was also significantly affected by diet in
248 mixed families ($F_{1,9.556}$=6.305, p=0.032) (Figure 4iii). In simple families, forewing area was
249 significantly affected by both adult type ($F_{1,8.644}$=1.030x10^3, p<0.001) and diet ($F_{1,8.764}$=17.120,
250 p=0.003). A significant interaction between adult type and diet was also found in simple families
251 ($F_{1,7.714}$=13.439, p=0.007); Tukey post hoc tests revealed that forewing area was significantly
252 reduced by the birch diet in females (p=0.003) but not in simple males (p=0.111) (Figure 4iv).

253 During this experiment, a single family (Family 2 from Table 1) was observed to have much
254 lower success rates of cocoon formation (52%) than all other families (93-100%). In this mixed
255 family, only 4 of 20 melanic males (20%) successfully formed cocoons before pupating,
256 compared to 14 of 21 simple males (67%) and 26 of 48 females (54%). These success rates were
257 compared using three 2x2 contingency Chi square tests. Melanic males were found to have a
258 significantly lower success rate of cocoon formation than both simple males (d.f.=1, χ^2=9.058,
259 p=0.003) and females (d.f.=1, χ^2=6.685, p=0.010), whereas simple males and females did not
260 differ significantly in their success rates (d.f.=1, χ^2=0.937, p=0.333).

261

262 **4. Discussion**

263 *4.1 Genetic basis of phenotypes*

264 This study showed that two distinct, pattern-based phenotypes are present in *M. disstria* moths,
265 and that one phenotype is measurably darker than the other. Although Lorimer (1979) proposed

that the melanic phenotype is sex-limited to males, this study found evidence that females carrying the melanic allele have slightly increased melanization compared to non-carriers. This suggests that melanism in *M. disstria* is only partially sex-limited: only males express the wing patterns and darkness characteristic of the melanic phenotype, but the melanic allele nonetheless affects wing melanization in female carriers. The results of this study also supported the hypothesis that polymorphic melanism in *M. disstria* is controlled by a pair of alleles at a single autosomal gene locus with the melanic allele being dominant (Lorimer 1979). Dominant melanic alleles at an autosomal locus have been shown to be responsible for many other documented cases of polymorphic melanism in moths. Although the sex-limitation is unusual, Kettlewell (1973) reported male-limited melanic phenotypes in three moth species (*Cycnia mendica*; *Hepialus humuli;* and *Lasiocampa quercus*, a member of the same family as *M. disstria* (Lasiocampidae)) .

The presence of the same phenotypes in Lorimer's (1979) study populations (Michigan, Minnesota, Indiana, and Alabama) and in the Alberta population used in this study indicate that this melanic polymorphism, and its sex-limitation, is widespread across much of the species' distribution. Furthermore, in this study the frequency of the melanic allele was 18%, while allelic frequencies of 4-19% were calculated from the sample sizes and phenotypic frequencies reported in Lorimer's (1979) study for Michigan and Indiana egg collections in 1976 and 1977. Given the geographical and temporal distances between the populations used in these two studies, allelic frequencies appear to be quite conserved across the species' distribution.

Indeed, the rarity of dominant melanic alleles may be conserved across species of moth as well. In the *Phigalia pedaria* moth, two melanic alleles are present (both dominant to the typical, or non-melanic allele) and occur at combined frequencies of 2-18% in rural areas across the British

Isles (Lees 1971). Cook *et al.* (2002) also report low frequencies (0-39%) of melanic phenotypes of three species of moths in non-industrial areas of Great Britain between 1974 and 1999. The melanic alleles are dominant in all three species, and although the authors did not report the allelic frequencies, the phenotypic frequencies are comparable to those reported by Lorimer (1979) (8-34%) and to that found in the present study (32%), indicating that allelic frequencies are also quite similar.

4.2 Melanism and larval development

The low quality larval diet tended to decrease body size and increase development time. Diet quality did not affect wing melanization, however, nor did it differentially affect the survival of melanic and simple (non-melanic) males. Melanic males were found to have both lower pupal mass and smaller wing area than non-melanic males regardless of diet treatment, indicating inherent developmental differences between the two phenotypes. Lorimer (1979) found no significant difference between mean pupal mass of melanic and non-melanic males; however, the larvae in her experiment were reared on prepared diet rather than on foliage, as was used here.

Trembling aspen is known to promote better larval development than prepared diet, resulting in significantly greater pupal masses (Colasurdo *et al.* 2009). It is probable that white birch, although a secondary host, also promoted better development than prepared diet. Indeed, mean pupal masses of melanic and simple males in mixed families were much higher in the current study (269mg and 283mg, respectively; diet treatments combined) compared to those reported by Lorimer (215mg and 204mg, respectively). Furthermore, Lorimer did not report any other information regarding larval rearing conditions in her experiment. Other factors may therefore have contributed to the different results obtained in these two experiments.

311 Size differences between melanic and non-melanic males could have an important impact on the
312 relative reproductive success of the two types of males due to differences in flight capabilities.
313 Male *M. disstria* must actively search for mates (Fitzgerald 1995); thus, their reproductive
314 success is largely dependent on their ability to fly until they find a receptive female. Flight
315 capabilities are determined by lipid content, thoracic muscle mass, and wing loading (Angelo
316 and Slansky Jr. 1984; Beck and Kitching 2007; Marden 2000). However, these traits can be
317 related to pupal mass and forewing area in different ways depending on resource allocation and
318 allometry (e.g. Boggs and Freeman 2005; Gunn and Gatehouse 1986; Gunn and Gatehouse 1993;
319 Muhamad *et al.* 1994; Shirai 1993). Further investigation is needed to determine whether the
320 observed reductions in pupal mass and adult forewing area are an advantage or a detriment to
321 flight capabilities, and therefore mating success, in melanic male *M. disstria* moths.

322 This study also found another indication of inherent differences between melanic and non-
323 melanic males. In one family, melanic males were found to have much lower rates of successful
324 cocoon formation compared to non-melanic males and females.The fact that this single family
325 had extremely low levels of cocoon formation in both diet treatments suggests that the larvae
326 were infected with a pathogen that interferes with silk production (e.g. Orr *et al.* 1994; Youssef
327 1974). Although normal adults eclosed from naked pupae in the lab, failure to spin a cocoon in
328 nature could result in the larvae falling to the ground and pupating unprotected. These pupae may
329 be more vulnerable to predation than those that successfully formed a cocoon. Melanic males,
330 being more susceptible to this pathogenic infection in the larval stage, might therefore suffer
331 greater pupal mortality than non-melanic males.

332 Although increased melanism has often been linked to increased immune responses in larvae by
333 virtue of both physical and chemical properties of melanin (e.g. Mikkola and Rantala 2010;

Wilson *et al.* 2001), this is not always the case. Goulson and Cory (1995) reported that at constant larval density, melanic *Mamestra brassicae* larvae were more susceptible to viral infection in the fifth instar than non-melanic larvae. Furthermore, no melanic polymorphisms were observed in the *M. disstria* larvae reared for this experiment. Without a link between adult and larval melanism, there is no reason to expect adult melanism to increase immune function in the larval stage.

The melanic phenotype must provide some advantage for male moths; otherwise, it would not persist in the species due to the costs of melanin production. In cooler weather, melanic males may have a thermoregulatory advantage over non-melanic males, increasing their chances of mating success (e.g. Punzalan *et al.* 2008b). It is also more difficult for visual predators such as birds to spot darker insects when they are flying in low light conditions (Majerus 1998), which could result in lower predation pressures on melanic males. In females, however, such advantages of the melanic phenotype may not be enough to overcome the costs of melanin synthesis. Although sex-limitation of melanic phenotypes is rare, it has been reported before (Majerus 1998) and may be favoured when males and females are under different sets of selection pressures (e.g. Ohsaki 1995). Flight is less important for the reproductive fitness of female *M. disstria* moths than for the males; rather, body mass is of utmost importance as it is tightly linked with fecundity (e.g. Parry *et al.* 2001). Indeed, although females carrying the melanic allele have increased melanization, it is not accompanied by the reduction in pupal mass and forewing area seen in males with the full melanic phenotype.

In this study, we have shown that polymorphic melanism is present in *M. disstria* moths. We have also provided support for the hypothesis that the melanic allele is autosomal and dominant (Lorimer 1979). Although the melanic phenotype is only expressed in males, this study showed

that the allele nonetheless increases melanization in female carriers. Furthermore, we found inherent developmental differences between the melanic and non-melanic phenotypes, which have important implications for male survival and reproductive success. Further investigation of the possible advantages and disadvantages of both phenotypes would clarify the effects these phenotypic differences may have on the population dynamics of the species as a whole, and could help explain why the melanic allele is often rare, yet maintained, in *M. disstria* and other polymorphic moth species.

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List of Figures

Figure 1. Wing samples for (a) patterned male, (b) simple male, and (c) (simple) female *M. disstria*. The proximal area of each wing is on the right and the distal area is on the left; these areas are delineated by the proximal and distal bars, respectively. Unlike simple males and females, patterned males have two pale bars adjacent to the dark proximal and distal bars and greater variation in shading along the top edge of the wing.

Figure 2. Mean (+/- S.D.) wing darkness (as greyscale values: low values are darker, high values are paler) of melanic (patterned) male, simple male, and female *M. disstria* moths on aspen and birch diets from (i) mixed and (ii) simple type families. Letters denote significance between adult types. No significant effect of diet treatment was found.

Figure 3. Mean (+/- S.D.) development time (in days) of melanic male, simple male, and female *M. disstria* moths in (i) mixed and (ii) simple type families and of moths reared on aspen and birch diets in (iii) mixed and (iv) simple type families. Letters denote significance.

Figure 4. Mean (+/- S.D.) pupal mass (in mg) of melanic male, simple male, and female *M. disstria* moths on aspen and birch diets in (i) mixed and (ii) simple type families. Mean (+/- S.D.) forewing area (in mm^2) of melanic male, simple male, and female M. disstria moths on aspen and birch diets in (iii) mixed and (iv) simple type families. Letters denote significance between adult types; stars denote significance between diet treatments.

List of Tables

Table 1. Results of Chi square tests for ratios of male phenotypes (Patterned=P; Simple=S) in mixed type families. Rows in bold indicate that there is no significant difference between the observed and expected frequencies for a given ratio. Alpha was increased to 0.10 to reduce the risk of committing a Type II error. All d.f.=1.

Family	P:S	Expected ratio	χ^2	p
1	23:9	**3:1**	**0.167**	**0.683**
		1:1	6.125	0.013
2	20:21	3:1	15.033	0.000
		1:1	**0.024**	**0.876**
3	21:28	3:1	27.000	0.000
		1:1	**1.000**	**0.317**
4	20:13	3:1	3.646	0.056
		1:1	**1.485**	**0.223**
5	11:11	3:1	7.333	0.007
		1:1	**0.000**	**1.000**
6	32:12	**3:1**	**0.121**	**0.728**
		1:1	9.091	0.003
7	11:15	3:1	14.821	0.000
		1:1	**0.615**	**0.433**
8	9:17	3:1	22.615	0.000
		1:1	**2.462**	**0.117**
9	23:15	3:1	4.246	0.039
		1:1	**1.684**	**0.194**
10	16:9	**3:1**	**1.613**	**0.204**
		1:1	**1.960**	**0.162**