Natural variation in the growth and development of *Protopiophila litigata* (Diptera: Piophilidae) developing in three moose antlers

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Abstract

In animals, the early-life environment influences growth and development, which can have lasting effects on life history and fitness into adulthood. We investigated patterns of growth, pupal development time, and their covariation, in wild antler fly larvae (*Protopiophila litigata*; Diptera: Piophilidae) of both sexes collected from three discarded moose antlers of varying size, chewing damage (used to infer relative age), and antler fly density. Males tended to be smaller and their pupation lasted longer than females. One of the antlers was highly attractive to adult antler flies, whereas the other two attracted few or none. Individuals from one antler of low attractiveness were smaller and took longer to eclose than individuals from either other antler, perhaps due to its high larval density. The relationship between body size and pupal development time also differed among antlers, being positively correlated in the most attractive antler and negatively correlated in the two other antlers.

Key Words – Body size, Development rate, Early-life environment, Life history, Pupation

The antler fly, *Protopiophila litigata* Bonduriansky (Diptera: Piophilidae), is a necrophagous fly that oviposits exclusively into discarded cervid antlers (Bonduriansky 1995). Over a summer, at least ten and up to a few hundred mated females may oviposit into an antler (C. Angell, personal observation). Antlers are patchily distributed and vary in quality, based at least partly on age (i.e. time since being shed). A given antler will support multiple cohorts of larvae for approximately four years, but it will gradually become unsuitable and stop attracting adults (Bonduriansky 1996). Other factors, such as the size and nutrient composition of antlers and larval density, are also likely to affect their quality as a natal environment. Although antler flies are a valuable model species for studying ageing in the wild (Bonduriansky and Brassil 2002), the life-histories of the larvae and pupae are not well characterized.

Both adult body size and timing of eclosion—and thus growth and development rate—are likely to be under directional selection in antler flies. Growth rate describes the rate of increase in size, while development rate describes the rate of cell differentiation (van der Have and de Jong 1996); these physiological processes are often correlated but are distinct. Larger males are more successful in agonistic intrasexual contests (Bonduriansky and Brooks 1999a) and are preferred by females as mates (Bonduriansky and Brooks 1998). Female fecundity is also correlated with body size in *P. litigata* (Bonduriansky and Brooks 1999b). Therefore, all else being equal, directional selection for rapid growth is likely in both sexes. Furthermore, theory predicts that fast development will be favored by both natural (Stearns 1992) and sexual selection (Andersson 1994) in seasonal organisms such as antler flies.

However, if growth and development are both energetically expensive, they may trade off with one another, both within and between life stages. For instance, individuals that allocate highly to growth and/or development as larvae may suffer slower development during the pupal stage. The ultimate body size of an individual is also constrained temporally by development rate, as fast larval development affords less time for growth. The optimal allocation to growth and development across

life stages will depend ultimately on the net fitness effect of the resulting life history, but this is beyond the scope of this note. Allocation patterns are likely to vary plastically among different larval environments. Differences in resource availability, competition, predation, or other factors may influence growth and development individually, as well as their covariation. Here we characterize differences in the larval growth and pupal development of wild antler flies of both sexes among three larval environments (natal antlers).

Three discarded moose (*Alces alces* [Linnaeus] [Artiodactyla: Cervidae]) antlers were collected from Algonquin Provincial Park, Ontario, Canada in May and June of 2017 and relocated to the Algonquin Wildlife Research Station. The antlers varied in size, attractiveness to adult flies, as measured by the average density of males on the antler surface at 13:00-14:00 between 7 July and 15 July 2017, and percent of surface area chewed by rodents and other animals (Table 1). Michael (1965) found that the extent of chewing damage to a white-tailed deer antler (*Odocoileus virginianus* [Zimmerman] [Artiodactyla: Cervidae]) is significantly related to the antler's age (time since shedding). Admittedly, chewing is an imperfect index of antler age, as he reported that antlers occasionally escaped chewing damage for up to three years; however, rodent abundance was extremely low for the duration of that study, which likely contributed to a lower frequency of chews than is typical (Michael 1965). In Algonquin Provincial Park, the degree of chewing damage on an antler is negatively correlated with the density of adult *P. litigata* (Bonduriansky 1996), which probably respond to biologically relevant sensory cues (e.g. odour) to determine antler quality.

Antlers A and B were located in May and larvae were collected from these antlers between 31 May and 11 July, while antler C was found on 22 June, so larvae from this antler were collected between 22 June and 11 July. For this reason, the absolute number of larvae collected should not be compared between them, but antler A yielded by far the fewest mean pupating larvae per day over the collection period (Table 1).

Mature piophilid larvae leave their larval environment (the bone matrix) to pupate when they detect the sound and moisture caused by rain (Bonduriansky 2002). Thus, *P. litigata* larvae were collected by hand from the antlers, either after rain or while simulating rain by spraying the antlers with water and drumming on them with sticks and pens. Each larva was placed individually in a 23 mm diameter plastic vial filled with 2-3 cm of coco peat ("Cocogro," Botanicare, USA) and capped with a foam plug, where they burrowed to pupate. Upon eclosion, adults were gently immobilized under a layer of plastic wrap following (Bonduriansky and Brooks 1997), sexed based on their terminalia (Bonduriansky 1995), and photographed under a dissecting microscope with an ocular micrometer. Thorax length was measured as a proxy for adult size, using ImageJ version 1.51 (Schneider et al. 2012). This measurement is repeatable and correlated with body size in *P. litigata* (Oudin et al. 2015). Voucher specimens were deposited in the Canadian National Collection of Insects, Arachnids, and Nematodes (Ottawa, Ontario, Canada).

We tested for differences in adult body size using Gaussian linear models in R 3.5.1 (R Core Team 2018). Previous research has reported a negative relationship between the date of eclosion and body size in this species (Bonduriansky and Brooks 1999a), so we included the date of pupation (i.e. emergence from the antler) as a covariate. Our initial model included fixed effects of antler, sex, and date of pupation, as well as all interactions, on thorax length. Pupation date was standardized to a mean of zero and a standard deviation of one prior to analysis (Schielzeth 2010). The model was simplified using a stepwise deletion procedure.

Second, we tested for differences in pupal development time (in days) with Gaussian linear mixed effects models, using the R package *lme4* (Bates et al. 2015). The initial model included fixed effects of antler, sex, and thorax length (standardized as described above), as well as all possible interactions. Pupae were kept in an uninsulated wood cabin subject to natural fluctuations in temperature depending on when they were collected, which likely influenced development rates. These fluctuations were highly variable and not monotonic, so date of pupation was included as a

random effect rather than a covariate. As above, the model was simplified using a stepwise deletion procedure. Significance testing for both analyses was performed with the R package *car* (Fox and Weisberg 2011) and partial residual plots were produced with the R package *visreg* (Breheny and Burchett 2017).

The three antlers in this study differed in a variety of ways (Table 1). Antler A was highly damaged, unattractive to adult flies, and yielded few mature larvae. Based on these combined lines of evidence, we consider antler A a relatively old, low-quality antler. In contrast, antler C was damaged very little, highly attractive, and yielded many larvae, suggesting a fresher, high-quality antler. Antler B was only slightly more damaged than antler C but nearly as unattractive to antler flies as antler A. It also had a high density of larvae and a much more skewed sex ratio at eclosion than the other antlers. Overall, 76% of collected larvae eclosed (86% from antler A, 74% from antler B, and 78% from antler C).

The reduced model for thorax length ($F_{3,251} = 52.1$, $P < 0.001$) included significant additive effects of antler ($F_{2,250} = 71.7$, $P < 0.001$) and sex ($F_{1,250} = 4.59$, $P = 0.033$), and no interactions. Antler B yielded smaller flies than the other antlers, and males were smaller than females (Fig. 1A). There was also a marginally nonsignificant trend for larvae pupating later to reach greater adult sizes (reduced model + pupation date: $\beta = 0.014 \pm 0.007$ SE, $F_{1,250} = 3.73$, $P = 0.055$).

The reduced model for pupal development time (likelihood ratio test: $\chi^2_6 = 40.2$, $P < 0.001$) included main effects of antler, sex, and thorax length, and the interaction between thorax length and antler. Antler ($F_{2,234.95} = 6.20$, $P = 0.002$) and sex ($F_{1,235.42} = 9.55$, $P = 0.002$) significantly influenced pupal development time, with males and flies from antler B pupating for longer (Fig. 1B). Thorax length was not correlated with pupal development time overall ($F_{1,234.18} = 0.78$, $P = 0.378$), but it significantly interacted with antler ($F_{2,235.18} = 3.71$, $P = 0.026$) such that larger flies pupated more slowly in antler C, but more quickly in antlers A and B (Fig. 1C-E). There was a marginally nonsignificant interaction between sex and thorax length, in which males displayed a

stronger negative correlation between thorax length and pupal development time than females (reduced model + sex × thorax: $F_{1,232.81} = 3.62$, $P = 0.058$). However, when one small male with an unusually long pupal development time was removed, this interaction no longer approached significance and the other results were qualitatively unchanged.

Here we show that growth and development in *P. litigata* differ significantly between the sexes and among natal antlers. Surprisingly, although antler B was not highly chewed (Table 1), individuals from this antler were smaller and pupated for longer than those collected from either other antler (Fig. 1A, B). Antler B was much less attractive to adult flies than would be expected given its level of damage (Fig. 2), suggesting it may actually be an old antler that largely avoided chewing by chance (Michael 1965). If this is the case, old age alone still cannot explain the differences between the flies from antler B and the similarly unattractive antler A. Antler B may have been a poor larval environment due to another factor beside antler age, such as its high larval density (Table 1) or unmeasured variables related to the moose the antler came from (e.g. nutrition, stress, resource allocation, etc.).

Interestingly, the correlation between body size (i.e. growth) and pupal development time differed among the antlers, which suggests the larval environment influences allocation among juvenile life-history traits. Flies from antler C, the least damaged and most attractive antler, eclosed more quickly if they were smaller (Fig. 1E), consistent with a growth-development trade-off. Conversely, individuals from antlers A and B eclosed more quickly if they were larger (Fig. 1C, D). Theory suggests that positive covariation between costly traits indicates high variation in resource acquisition, whereas negative covariation indicates variation in allocation (van Noordwijk and de Jong 1986). Since large body size (i.e. greater growth) was correlated with faster pupal development in the two less attractive, and likely lower quality, antlers, perhaps competitive resource acquisition is more important in a depleted environment, while variability in allocation was only apparent in the attractive, and plausibly resource-rich, antler C.

Surprisingly, although a previous mark-recapture study of *P. litigata* reported that body size decreased significantly in later-emerging males (Bonduriansky and Brooks 1999a), we found that body size did not change significantly with pupation date. This discrepancy may be due to the fact that our analysis takes into account among-antler differences, whereas Bonduriansky and Brooks (1999a) measured body size at the whole population level. If flies from low-quality antlers are generally both smaller and slower-developing, as in antler B (Fig. 1A, B), this effect may drive population-level changes in body size through the season.

Finally, we note that the sex ratio at eclosion was notably male biased in antler B only (Table 1). Assuming an even sex ratio at oviposition, this suggests sex differences in mortality in this species during the larval or pupal stage alongside the sexual dimorphism in other juvenile traits reported here. Specifically, females may be less resilient to antler B's high larval density. This single observation is far from conclusive, but it suggests a sex-specific effect which occurs prior to maturity. Alternatively, the sex ratio of eggs may have been male biased initially. Male antler flies are smaller than females, and presumably require less energy to grow, so mothers could favor male offspring when ovipositing into a high density or depleted environment (cf. Wiebe and Bortolotti 1992).

Overall, these results demonstrate that growth and development differ among larval environments in *P. litigata*. Antler age, larval density, and other factors likely all contribute to environmental quality, and this study does not have sufficient independent replication to distinguish between the effects of each. The genetic background of the eggs oviposited into each antler may also have contributed to differences in growth and development among antlers. This work represents an important first step in characterizing natural variation in life-history traits in antler flies, and a longitudinal study of several antlers through time would be even more informative for distinguishing the factors that influence this variation. Given the antler fly's suitability for ageing

research, future work should also investigate how antler quality affects lifespan and reproductive senescence throughout adulthood.

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Table 1. Attributes of three moose (*Alces alces*) antlers containing larval antler flies, *Protopiophila litigata*.

Antler	Surface area (cm^2)	Chewing damage	Average male density (cm^{-2})	Number of larvae collected[a]	Mean number of larvae per day	Proportion of flies male
A	840	12%	0.00	22	0.54	0.53
B	952	1.4%	0.04	277	6.8	0.68
C	874	0.69%	4.4	82	4.3	0.54

[a]Because not all larvae eclosed successfully and a few escaped before they could be measured, the statistical analyses included only 19, 176, and 57 individuals from each antler, respectively
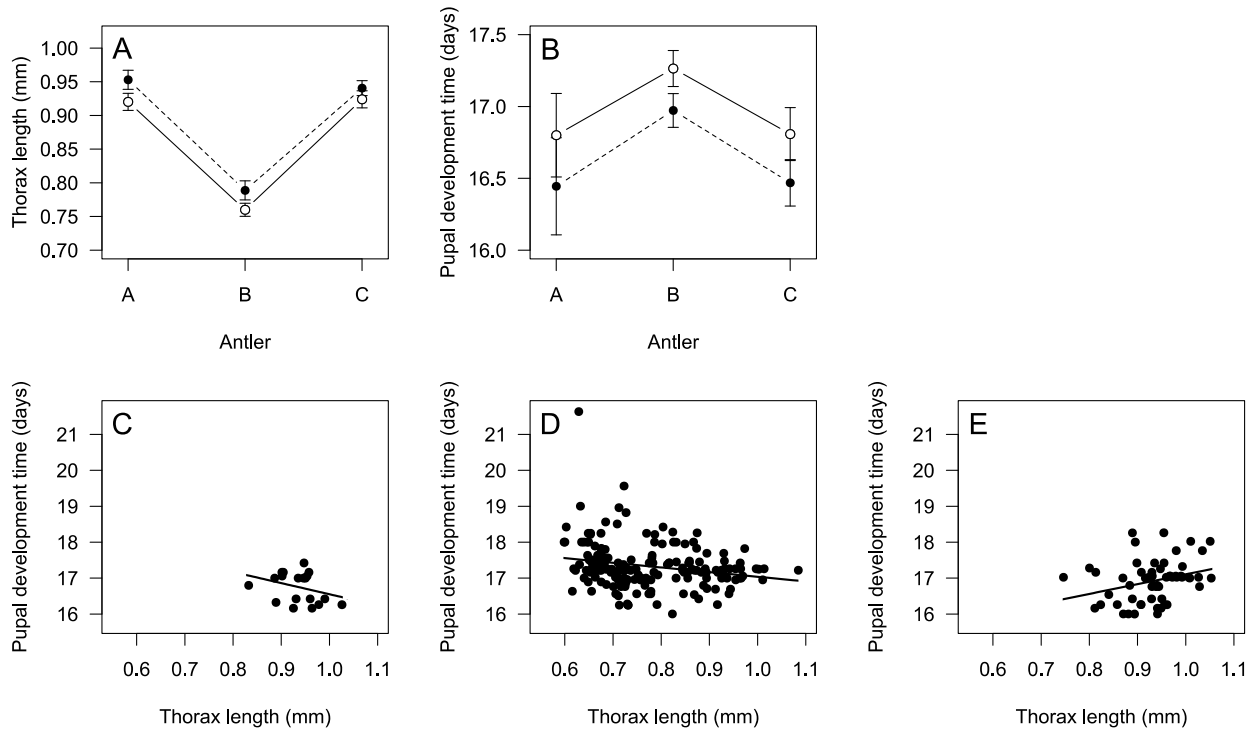
Fig. 1. Factors affecting growth and development of *Protopiophila litigata*. **A**, Mean (± SE) thorax length among antlers and between sexes. **B**, Mean (± SE) pupal development time among antlers and between sexes. In panels A and B, hollow circles are males and filled circles are females. **C-E**, Partial residuals plots showing the partial effect of thorax length on pupal development time separately in antler A (panel **C**), antler B (panel **D**), and antler C (panel **E**).
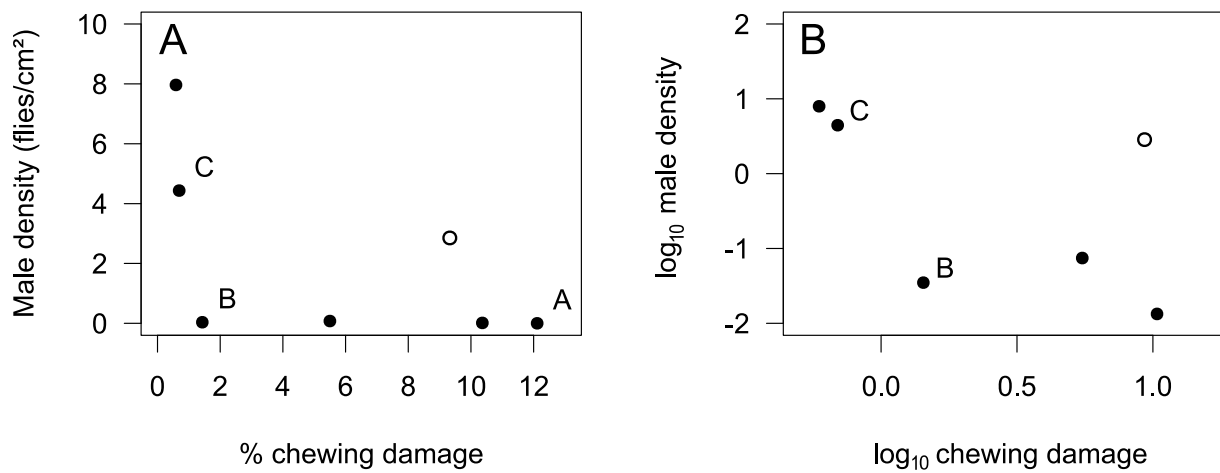
Fig. 2. The relationship between the proportion of antler surface area damaged by animal chewing and mean male *P. litigata* density (antler attractiveness) on seven moose antlers in 2017. Data are shown both untransformed (**A**) and $\log_{10}$ transformed (**B**). Previous work by Bonduriansky (1996) demonstrated a strong correlation between these variables on a log-log scale (r = -0.97). Letters correspond to antlers in Table 1. Antler A does not appear on the $\log_{10}$ plot as it had a male density of zero. One antler (hollow circle) had been stored inside a shed, away from access by antler flies, for a year prior to the study.