NOTE / NOTE

Locomotion is not influenced by denticle number in larvae of the fruit fly *Drosophila melanogaster*

Mark J. Fitzpatrick and Evelyn Szewczyk

Abstract: Denticles are small projections on the underside of larval fruit flies that are used to grip the substrate while crawling. Previous studies have shown that (*i*) there is natural variation in denticle number and pattern between *Drosophila melanogaster* (Meigen, 1830) and several closely related species and (*ii*) mutations affecting denticle morphology have negative effects on locomotory performance. We hypothesized that there would be a correlation between denticle number and locomotory performance within populations of *D. melanogaster*. Despite finding considerable variation in denticle number, we found no correlation between denticle number and three measurements of larval locomotion: speed, acceleration, and absolute turning rate.

Résumé : Les denticules sont de petites excroissances sur la paroi ventrale des larves de la mouche à fruit utilisées pour s'agripper au substrat durant la reptation. Des études antérieures ont montré que (*i*) il existe des variations naturelles dans le nombre et la répartition des denticules entre *Drosophila melanogaster* (Meigen, 1830) et d'autres espèces procheset que (*ii*) les mutations qui affectent la morphologie des denticules ont des effets négatifs sur les performances locomotrices. Notre hypothèse de travail est qu'il y a une corrélation entre le nombre de denticules et les performances locomotrices dans un même population de *D. melanogaster*. Bien qu'il y ait de nettes variations du nombre de denticules, nous n'avons pas pu démontrer de corrélation entre le nombre et trois descripteurs de la locomotion de la larve : la vitesse, l'accélération et le taux absolu de changement de direction.

Introduction

Locomotion is critical during the life history of many organisms ranging from animals to protists (Alexander 1982; Dickinson et al. 2000). Comparative analyses have revealed that differences in locomotion seem to be linked with evolutionary changes in morphology (e.g., Losos 1990; Full 1994). Morphological features known to affect locomotion include mass, bone structure, musculature, and various external structures (e.g., Inestrosa et al. 1996; Donovan and Carefoot 1997; Quillin 1999; Aerts et al. 2000; Russell and Bels 2001; Wilga and Lauder 2001; Brana 2003). Laboratory-induced mutations are routinely studied with regard to their influence on locomotion (Hurd and Saxton 1996; Inestrosa et al. 1996; Yang et al. 2000; Ainsley et al. 2003). However, these mutations often cause severe aberrations in the animal that (*i*) do not occur in nature and (*ii*) may affect many other factors in addition to the trait of interest. Conversely, naturally existing variation in morphology found within a species is rarely studied with respect to its influence on locomotory performance (but see Fitzpatrick et al. 2003).

Many larval dipterans, like various other limbless animals, crawl by means of peristaltic waves traveling from the anterior to the posterior end (Heffernan and Wainwright 1974; Green et al. 1983; Berrigan and Pepin 1995). In larval fruit flies, denticles (also referred to as chaetae), small toothlike projections, are used to grip the substrate, enabling the larvae to move forward. Sucena and Stern (2000) showed that remarkable natural variation in denticle number exists between species that are closely related to *Drosophila melanogaster* (Meigen, 1830). However, natural variation in denticle number within a single species has not been documented. In addition, the role of denticle number in locomotion has not yet been addressed either within or between species. The locomotion of larval *D. melanogaster* is compromised by mutations that mildly affect denticle morphology and that are presumed to influence the ability of the larva to grip the substrate (Inestrosa et al. 1996). Here we quantify several measures of locomotion, which are then correlated with denticle

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number within a natural population of fruit flies. We hypothesized that larvae having more denticles might exhibit enhanced locomotion owing to a better ability to grip the substrate.

Materials and methods

Study population

All larvae used in this study were the descendants of a wild population of *D. melanogaster* collected in September 2002 from an abandoned apple orchard found on the campus of the University of Toronto at Mississauga, Ontario, Canada. Fruit flies were reared on 40 mL of standard dead yeast, sucrose, and agar medium in 170-mL plastic urine containers (B352, Simport Plastics, Beloeil, Quebec, Canada). The population was maintained on a 12 h light : 12 h dark cycle at 22 ± 1 °C. The animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Locomotory behaviour

All larvae were in the third instar (96 ± 2 h old) at the time of testing (standard procedure; Pereira et al. 1995). Individual larvae were placed in the centre of a 14.5 cm diameter petri dish containing a moist agar substrate (50 mL distilled H_20, 0.67 g agar, and 0.13 g powdered charcoal). The charcoal provided a dark background, allowing the larvae to be visualized as solid white objects. The agar provided a nonnutritive substrate that allowed for movement without causing desiccation of the larvae (see also Green et al. 1983; Caldwell et al. 2003). Larvae were allowed 30 s to acclimate to the arena before testing. Locomotory behaviour was recorded in 3-min digital movies via the software Northern Eclipse (Empix Imaging Inc., Mississauga, Ontario, Canada) using a custom digital camera (Empix Imaging Inc.) with a 1280 pixel × 1024 pixel chip equipped with a 55-mm macro lens (Nikon). All recordings were made on 3 March and 4 March 2003. Movies were quantified using the Dynamic Image Analysis System (DIAS®, Solltech Inc., Oakdale, Iowa, USA) for Macintosh. The following measurements were obtained for each larva: speed (µm/s), acceleration (µm/s^2), and absolute turning rate (deg./s).

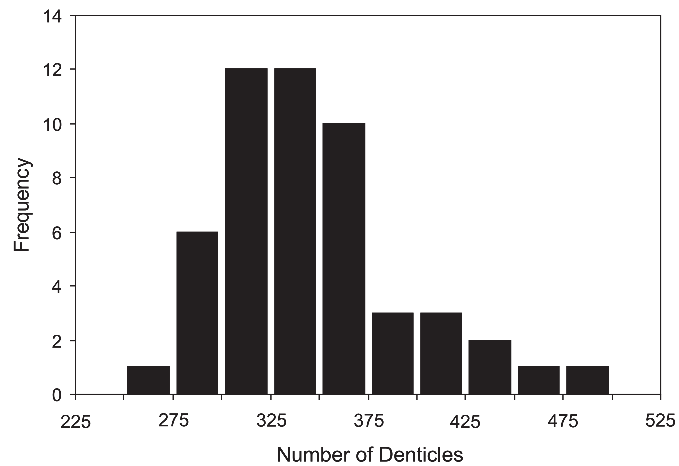
Denticle measurement

Immediately after the locomotory behaviour assay, the larvae were euthanized by placing them in boiling water for approximately 3 s. This caused the bodies to elongate, making the denticles easier to visualize. The larvae were placed under a dissecting microscope with the ventral side facing upwards. A digital image was taken of the first abdominal segment from the anterior end using the software Northern Eclipse and a QICAM monochrome 10-bit digital camera (32-0030B-183, QImaging, Canada). We used the first abdominal segment because (*i*) species-level variation in denticle number has been reported at this segment (Sucena and Stern 2000) and (*ii*) it appears to be used to grip the substrate during locomotion. From these images we manually counted the number of denticles. Denticles were measured blind to the locomotion scores.

Table 1. Summary statistics of denticle number and locomotory scores of *Drosophila melanogaster* (N = 49).

Characteristic	Range	Mean	SD
Number of denticles	290–504	369	46
Speed (µm/s)	0.728–4.162	2.399	0.688
Acceleration (µm/s^2)	0.023–1.982	0.751	0.509
Absolute turning rate (deg./s)	0.0481–0.8686	0.2787	0.1556

Fig. 1. Natural variation in denticle number in a population of *Drosophila melanogaster* (N = 49).



Statistical analysis

Wilk–Shapiro rankits were used to assess normality using the software Statistix 2.0 (Analytical Software, Tallahassee, Florida). Values in excess of 0.9 were considered to be sufficiently normal. Absolute turning rate was log transformed to achieve normality. The remaining variables were all significantly normal without transformation. Pearson's correlation was used to assess the relationship between locomotion and denticles, using SYSTAT® (SPSS Inc. 1998).

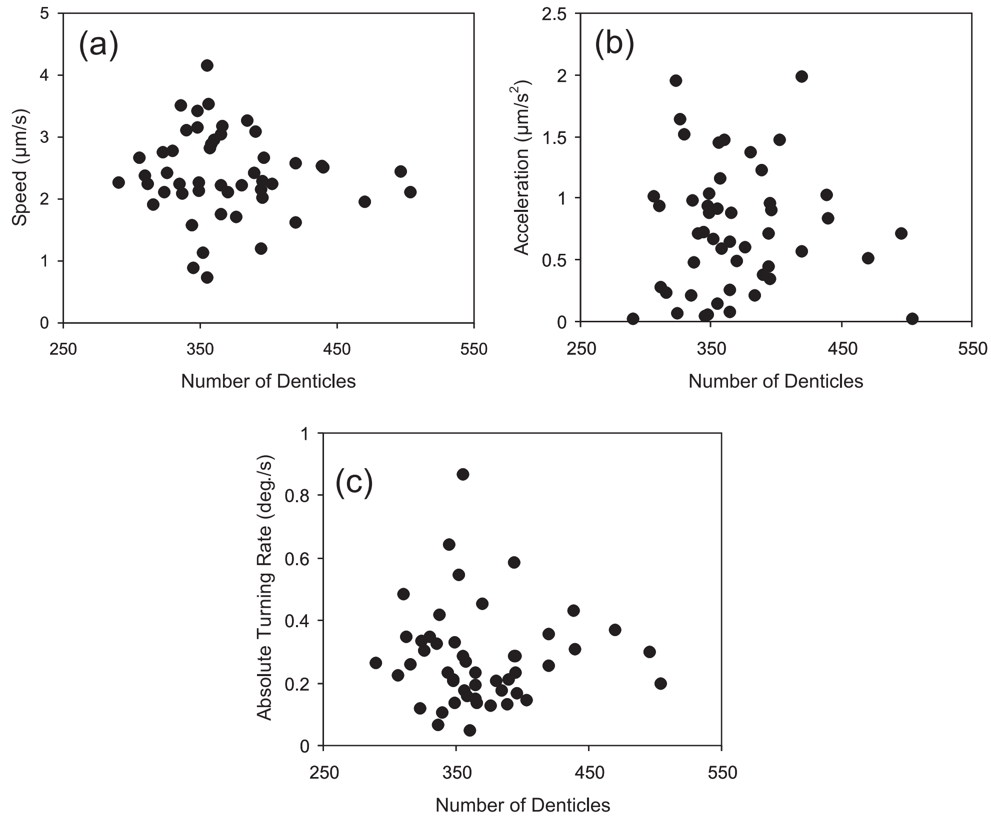
Results

Summary statistics for all variables are presented in Table 1. The number of denticles ranged from 290 to 504 (N = 49, mean = 369, SD = 46; Fig. 1). All three measurements of locomotion examined in this study were not influenced, positively or negatively, by the number of denticles (Fig. 2). Denticle number was not correlated with larval speed (r = –0.086, p = 0.559), acceleration (r = –0.007, p = 0.964), or absolute turning rate (r = 0.040, p = 0.783). A post-hoc power analysis revealed high power (in excess of 0.75) for all three comparisons to detect a medium effect or greater (Coen 1988; Faul and Erdfelder 1992). The three measurements of locomotion were highly intercorrelated. Speed was positively correlated with acceleration (r = 0.374, p = 0.008) and negatively correlated with turning rate (r = –0.581, $p <$ 0.001). Acceleration was negatively correlated with turning rate (r = –0.321, p = 0.024).

Discussion

Although we found natural variation in the number of denticles within our population, denticle number was not

Fig. 2. Correlations of natural variation in denticle number with three measures of locomotion: (*a*) speed (r = –0.086, p = 0.559), (*b*) acceleration (r = –0.007, p = 0.964), and (*c*) absolute turning rate (r = 0.040, p = 0.783) (N = 49).



correlated with measures of locomotory performance in our laboratory trials. In this experiment we measured natural variation in denticle number, whereas Inestrosa et al. (1996) found that a laboratory-induced mutation that mildly affected the morphology of the denticles had a negative influence on locomotion. In both our experiment and that of Inestrosa and colleagues, agar was used as the substrate. To the best of our knowledge, the existence of natural variation in denticle morphology and its role in locomotion have not yet been investigated in any species of *Drosophila*. Future experiments looking at other aspects of denticle morphology will be beneficial.

Sucena and Stern (2000) found that *Drosophila sechellia* (Tsacas and Bachli, 1981) have very few denticles per segment in comparison with related species of the *D. melanogaster* species group. This species-level variation in denticle number may be attributable to different environments occupied by larvae of the different species. An interesting future experiment would be an investigation of whether a correlation between locomotion and denticle number and morphology is seen at the species level. Moreover, mutations in the gene *shavenbaby* cause a drastic reduction in denticle number in *D. melanogaster* (very similar to the number found in *D. sechellia*) (Delon and Payre 2004). It will be interesting to investigate whether the locomotory performance of larvae carrying the *shavenbaby* mutation is altered.

Unlike studies using isogenic strains of *D. melanogaster*, this experiment relies on substantial natural variation in all traits measured in our analysis (see Fig. 2); therefore, we used a natural population of *D. melanogaster*. As expected, the range of variation in locomotion scores that we report for our population is larger than the range of measurements we obtained from an isogenic Oregon-R strain of *D. melanogaster* that was analyzed under the same conditions (e.g., speed: Oregon-R, N = 19, mean = 0.996 µm/s, SD = 0.236; data for the natural population are reported in Fig. 2). Thus, we believe that finding considerable variation in denticle number and the assorted measures of locomotion validates our use of the natural population.

Finding no correlation between denticle number and locomotion has important implications for our interpretation of the foraging behaviour of larval fruit flies (reviewed in Fitzpatrick and Sokolowski 2004). Locomotion is an integral component of larval feeding and foraging behaviour. Food is shoveled into the mouth as larvae move through a patch of food (Sokolowski et al. 1984), and larvae rely on locomotion to locate new patches of food (Sokolowski 1980). Although allelic variation at the *for* locus accounts for a large amount of the natural variation in foraging behaviour, there still remains unexplained variation in this behaviour at the population level. The current study suggests that naturally existing variation in denticle number does not influence the locomotory component of foraging. The remaining variation might be explained by factors such as humidity, temperature, light, viscosity of food, additional morphological features, and allelic variation in unidentified genes.

We feel that it is important to integrate information from all levels of analysis to fully understand the role of morphology in locomotion. Comparative analyses can elucidate major evolutionary changes. Studying mutants can reveal whether morphological structures are important for normal locomotion, even though these mutants are often unlikely to exist in nature. Finally, investigating the effect of within-species morphological variation on locomotion will help us understand the microevolutionary forces shaping the behaviour of the animal.

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