

# Protein variation in the larval diet directly effects the development time and adult size of *Culex pipiens f. molestus*, but maternal effects are minimal

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**Abstract:** The urban adapted mosquito *Culex pipiens f. molestus* lives predominately underground in human-made landscapes including sewers, subway tunnels and other subterranean locations. Females of this mosquito obligately lay a small batch of eggs prior to seeking a blood meal (autogeny). **This trait suggests that these mosquitoes need access to a source of protein in their larval diet.** However, it remains unclear what proportion of their diet must be made up of protein in order for these mosquitoes to develop successfully and then lay an autogenous batch of eggs. It is also unknown if variation in protein access predominately effects final adult size or the rate of larval development. **This research aimed to investigate how larval diet, particularly protein content, influences rate of development and adult size** in a lab-reared population of *Cx pipiens f. molestus* derived from New York City. We also investigated subsequent maternal effects of protein access on adult size in a second generation. This research involved rearing large batches of larvae using combinations of whole-wheat flour and liver protein, plus a control group fed on fish flakes. The protein content of these diets ranged from 13% to 70%. Surprisingly, at almost all protein levels mosquitoes developed to adults at the same rate. However, **there was a subtly but clear effect of protein content on adult size.** Most adult females from all diet treatments successfully laid an autogenous egg batch. The offspring of these females showed no maternal effects of diet variation. This research reveals that in contrast to current assumptions, these mosquitoes likely do not need a large source of protein in their natural habitat to develop successfully and lay autogenous eggs. However, variation in protein access may have more subtle fitness effects not observed in this study.

## Background

- *Culex pipiens f. molestus* live predominately underground in human-made landscapes including sewers, subway tunnels and other subterranean locations (Byrne et al, 1999; Kassim et al, 2011).
- This species is of special concern with its close proximity to humans and the potential to carry mosquito borne diseases (Fonseca et al 2004; Kassim et al, 2011; Nelms et al, 2013).
- Females of this mosquito obligately lay a small batch of eggs prior to seeking a blood meal (autogeny) (Kassim et al, 2011; Kassim et al, 2012)
- Protein is an important part of the diet for most mosquito larvae (Merritt et al, 1992) indicating this nutrient may be essential for autogenous mosquitoes (Kassim et al, Feb 2012).
- It remains unclear what proportion of their diet must be made up of protein in order for these mosquitoes to develop successfully and subsequently lay an autogenous batch of eggs.
- It is also unknown if variation in protein access predominately effects final adult size or the rate of larval development.
- We asked the question: how does access to protein in the larval diet influence rate of development and adult size in a lab-reared population of *Cx. pipiens f. molestus*.
- We also sought to answer the question of whether maternal access to protein influences size in a second generation.

## Methods

- We used whole wheat flour, liver powder and combinations of the two to create five different larval diets.
- These were: 100% flour (13%), 75/25% flour-liver powder (27.25%), 50/50% flour-liver powder (41.5%), 25/75% flour-liver powder (55.75%) and 100% liver powder (70%).
- Our control diet was common fish flakes consisting of ~47% of curd protein
- 19 egg rafts were collected from adults, and the hatching larvae from each raft were divided into groups of 6 individuals for each of the six diets
- Larvae were fed 3.2mg per individual every other day of selected diet for all instar stages
- Larvae remained in an incubator at a constant 23 C° and 12:12 (L:D) light cycle.
- The number of individuals and sex ratios were documented each day until all pupae had emerged.
- Emerging females were allowed to breed with unrelated males and collected later for analysis.
- Mosquito adults were sustained on a 10% glucose solution
- Second generation larvae were raised in the exact conditions except all individuals were fed the control diet of fish flakes.

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## F1 male wing length differed from lowest to highest while F2 did not

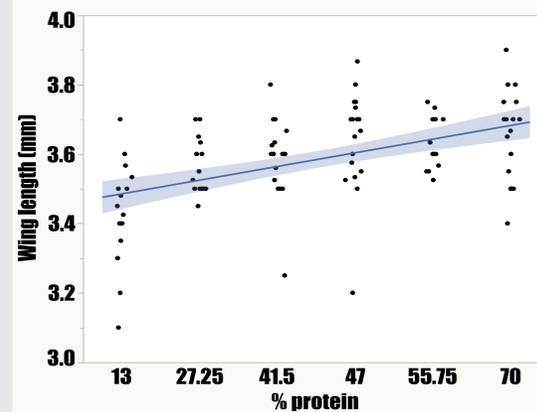


Fig 1: A linear regression was used to assess the correlation between male wing length and the protein content in the larval diet for males R = 0.287 (95% CI) ANOVA (df = 5; P = <0.0001)

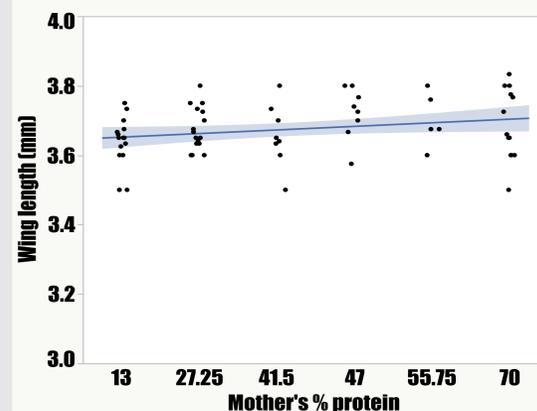


Fig 2: A linear regression was used to assess the correlation between second generation male wing length and the protein content of their mothers. These males were all fed the same control diet of 47% protein. R = 0.069435. ANOVA (95% confident interval; df = 5; P = 0.0037)

## F1 female wing length differed from lowest to highest while F2 did not

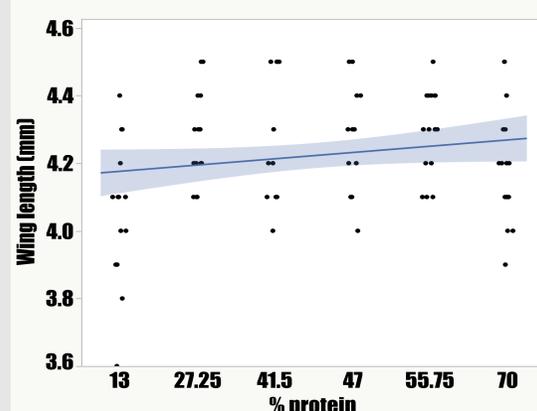


Fig 3: A linear regression was used to assess change between wing length with increasing protein content for F1 females. R = 0.214932. ANOVA (95% confident interval; df = 5; P = 0.0011)

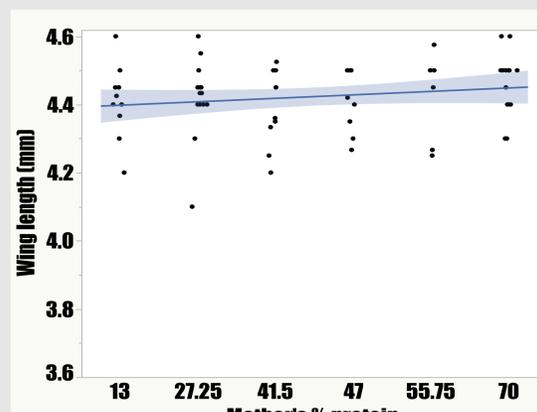


Fig 4: A linear regression was used to assess change between wing length with increasing protein content for F1 female's female offspring that were fed the same control diet of 47% protein. R = 0.032778. ANOVA (95% confident interval; df = 5; P = 0.4473)

## All adult first-generation mosquitoes emerged even on minimal protein diets. Second generation male offspring all developed at similar rates, irrespective of how much protein their mothers received in their larval diet

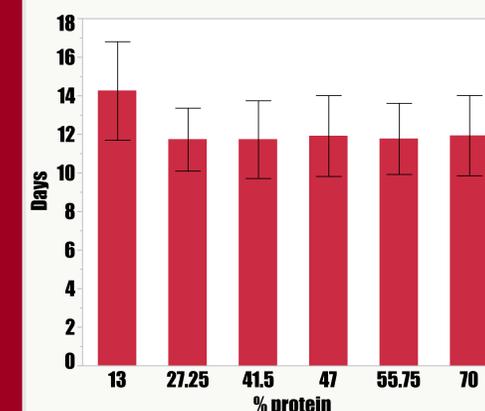


Fig 5: Mean time from first feeding to adult emergence for F1 generation. ANOVA (95% confident interval; df = 5; P = <0.0001)

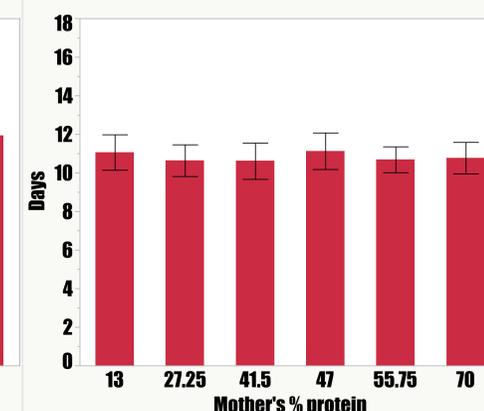


Fig 6: Mean time from first feeding to adult emergence for F2 generation. ANOVA (95% confident interval; df = 5; P value = 0.1160)



Fig 7: Adult mosquito housing with oviposition trays



Fig 8: Larvae housing containers of 1,000ml with 500ml of DI water in an environmental control chamber

## Conclusions

- Protein within the larvae's diet can influence time to emergence with individuals at the lowest protein percent (13%) emerging on average 2 days later.
- More significantly, *Cx. pipiens f. molestus* adults showed a significant decrease in size with decreasing protein with larvae at the lowest protein access being the smallest.
- Although protein within the larvae's diet can influence time to emergence and final adult size, all females of all treatments were able to produce viable offspring when offspring were subjected to diets rich in protein with no significant difference between F2 larvae.

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