Pupal size as a proxy for fat content in laboratory-reared and field-collected *Drosophila* species

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Introduction – Insect body size

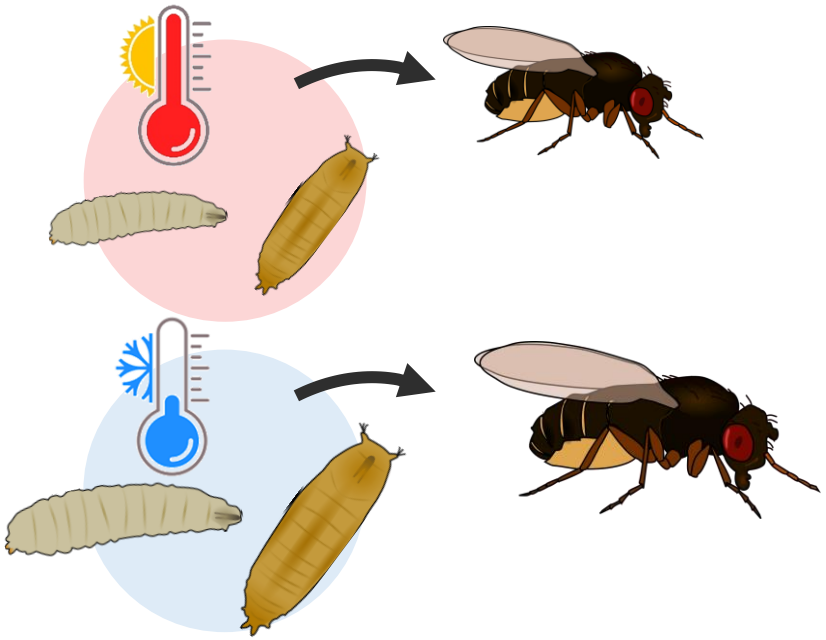
Size is a key trait in animals that can show high intra-specific variation

In insects, size is greatly influenced by environmental factors

Examples:

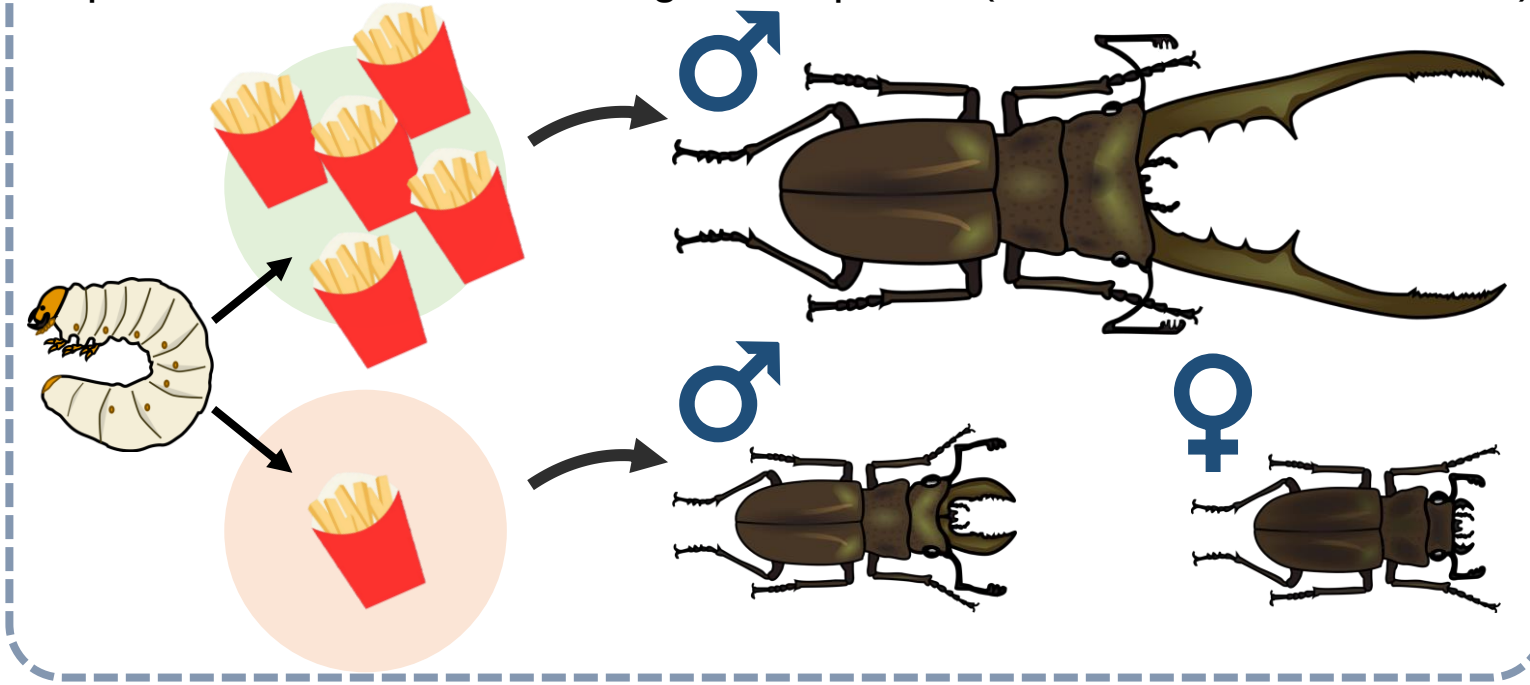
Temperature-size rule (ectotherms)

Developmental temperature in *Drosophila subobscura* (Kari & Huey, 2000, J Therm Biol)



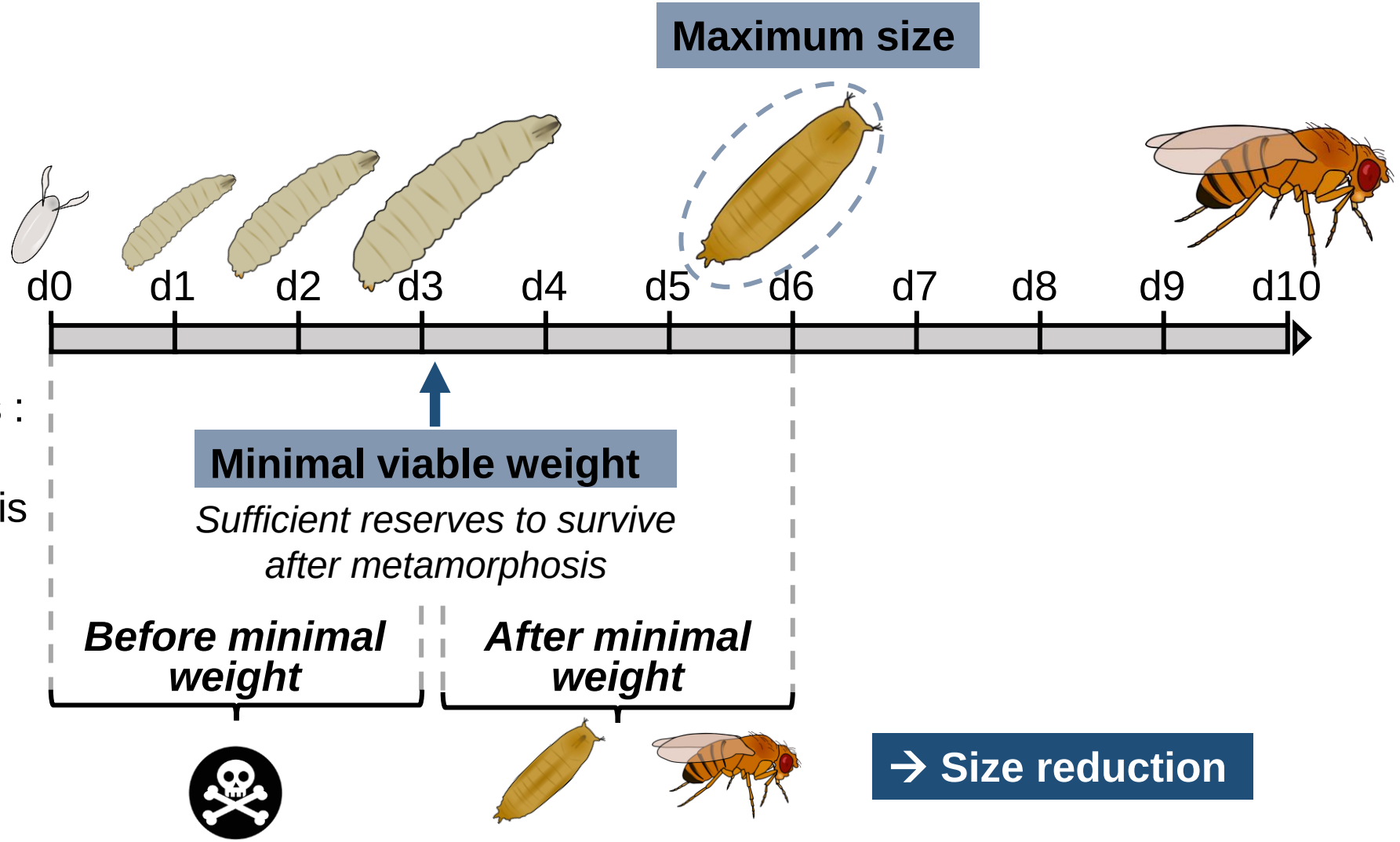
Resources during development

In stag beetles (Coleoptera, Lucanidae) : Variation in male size depends on food intake during development (Otoh et al, 2011, PLoS one)



Introduction – Insect body size

Insects do not grow as adults: size is determined during juvenile stages



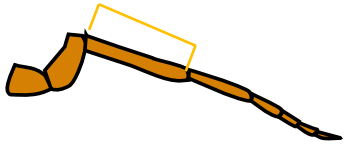
In holometabolous insects :
Critical steps for the initiation of metamorphosis

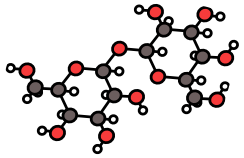
(Mirth & Riddiford, 2007, BioEssays; Beadle et al, 1938, Biol Bull)

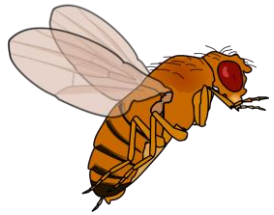
Introduction – Insect body size

Body size correlates with many other traits (**allometry**)

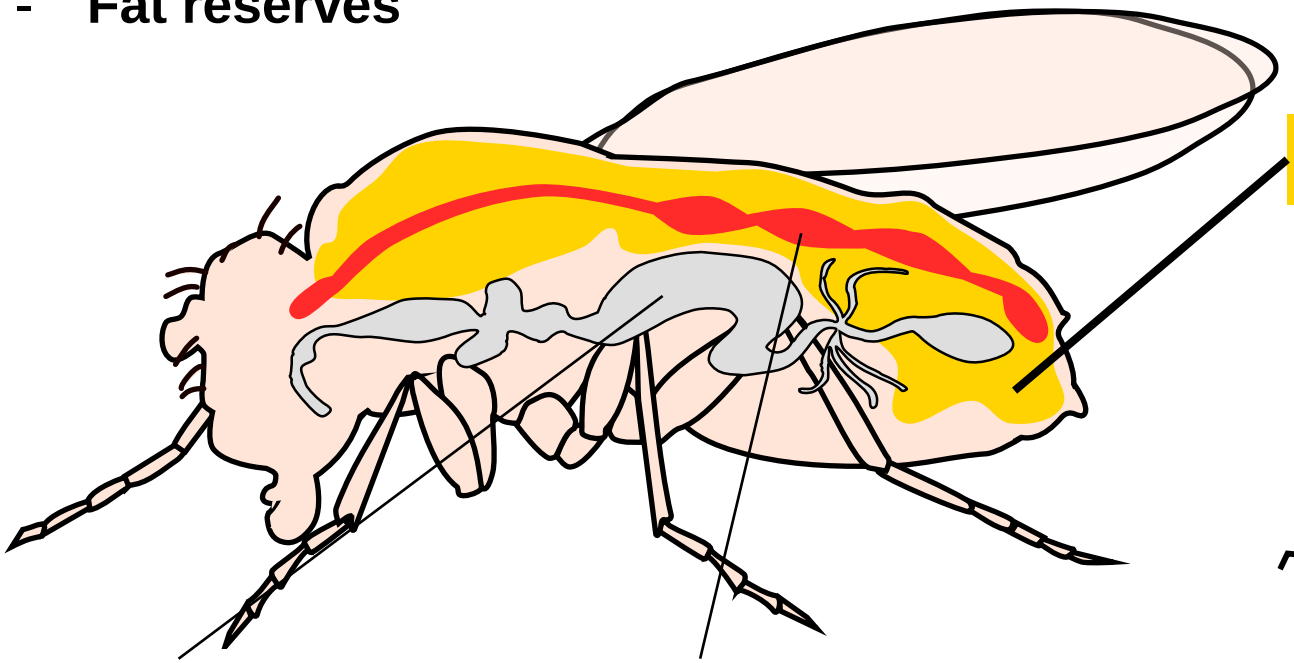
- Relative dimension of body parts; Physiological traits; Behavioral traits; (…)







- **Fat reserves**

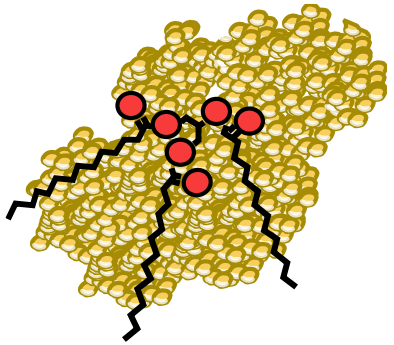


Digestive tract

Heart (dorsal vessel)

Fat body

= Triglycerides (i.e., fat)



Fat reserves affect

- Metamorphosis
- Longevity
- Fecundity
- Stress resistance
- Starvation resistance
- Immunity
- Overwintering
- …

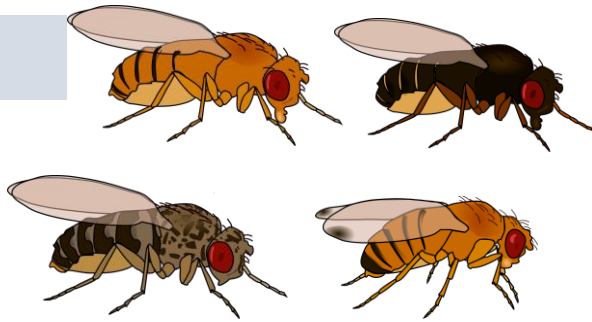
Fitness

(Gayon, 2000, Am Zool; Lease & Wolf, 2011, Physiol Entomol)

Problematic – the case of *Drosophila* flies

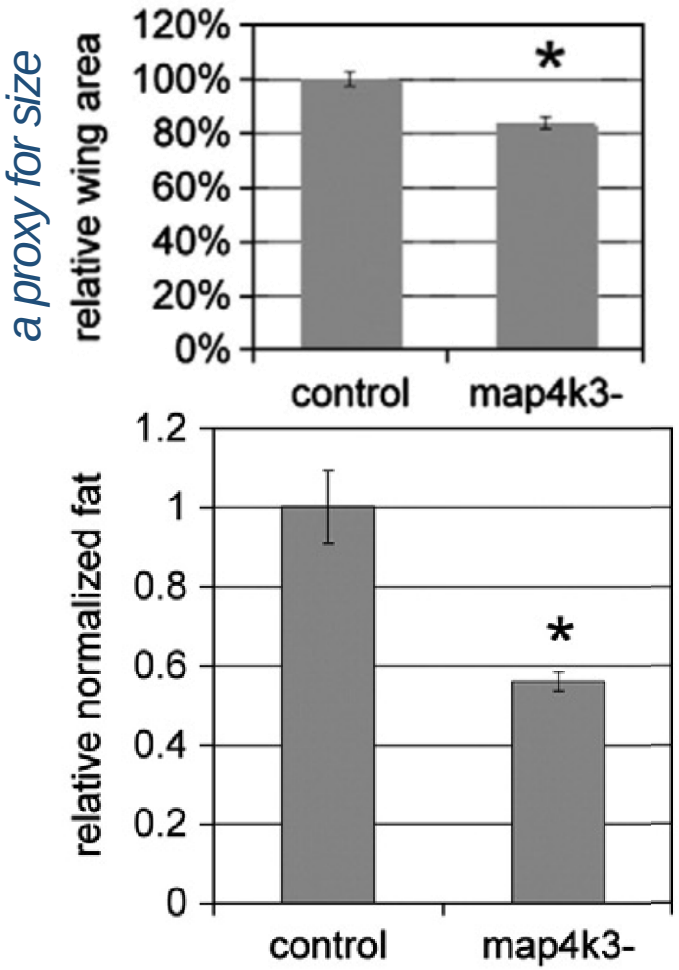
Larger individuals should have higher fat reserves and consequently higher fitness

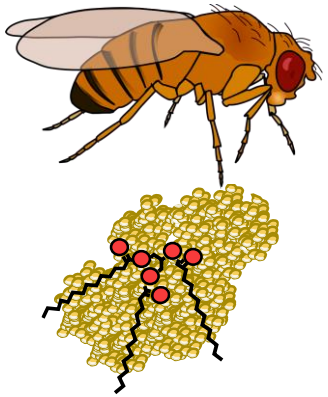
However, empirical data are lacking for many taxa, including *Drosophila sp.*

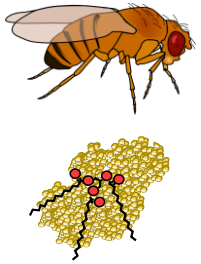


In *D. melanogaster,* relatively few studies show a link between size and fat content

- Bryk et al, 2010, Dev Biol:



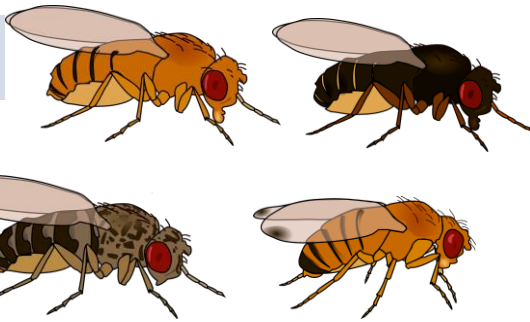




Problematic – the case of *Drosophila* flies

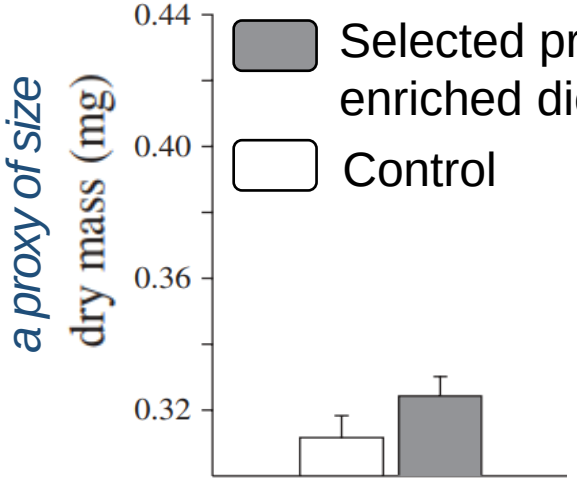
Larger individuals should have higher fat reserves and therefore higher fitness

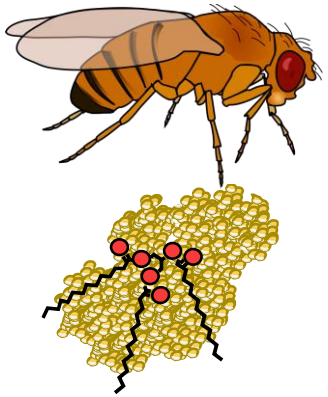
However, empirical data are lacking for many taxa, such as *Drosophila sp.*

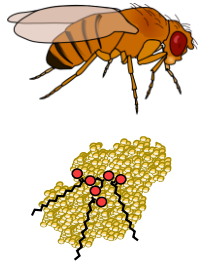


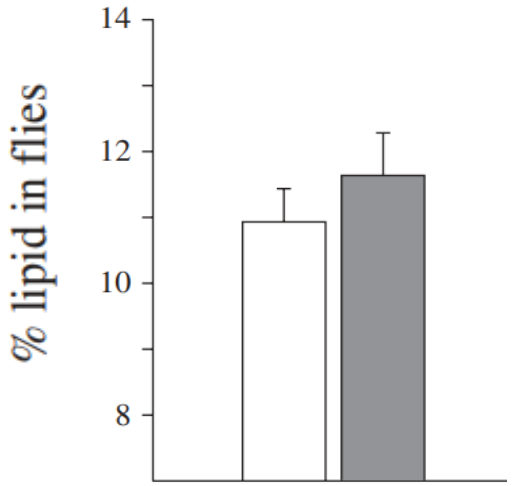
In *D. melanogaster* relatively few studies show link between size and fat content

- Kristensen et al, 2011, Biol Let:









No data to show a direct link between size and fat content

No data on juvenile stages (where the maximal size is fixed)

Aims of the study:

1-Test the relationship between size and fat content in *Drosophila* pupae

Expectation: correlation size / fat content

2- Establish a non-invasive method to estimate pupal fat content

Methods – Relationship between pupal size and fat content

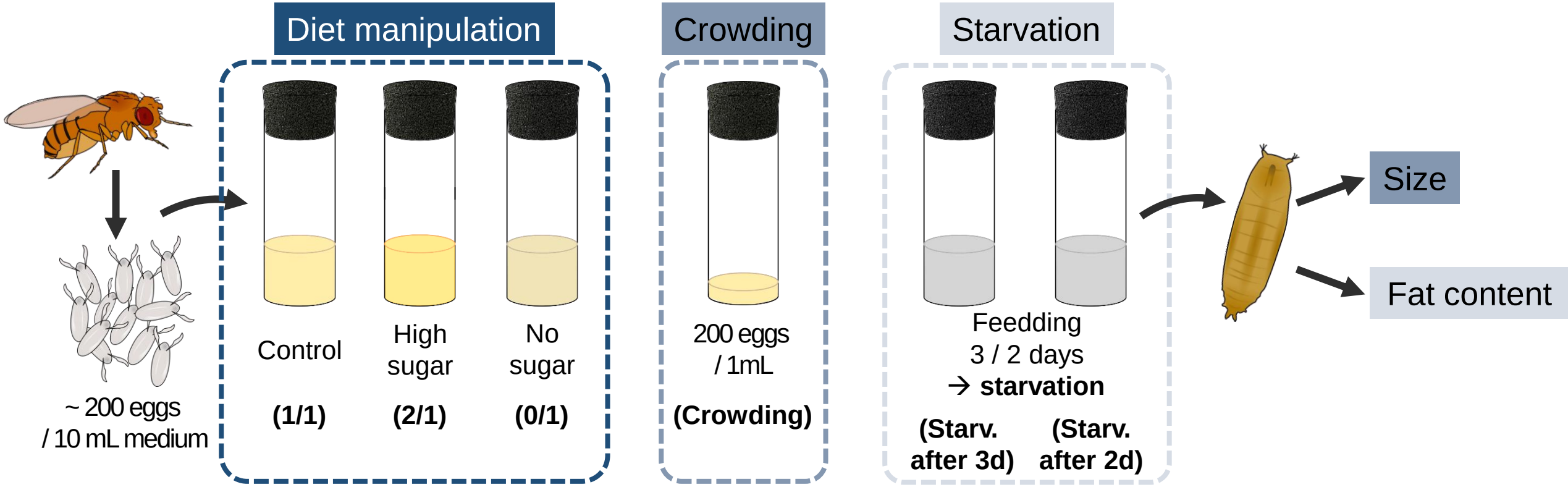
Work plan

1- Manipulate developmental conditions of *D. melanogaster* larvae to produce variation in size and fat content

2- Test the relationship between pupal size and fat content

a - In laboratory-reared *D. melanogaster*

- Laboratory-reared *D. melanogaster*



(1/1 = 1 part sugar, 1 part yeast)

Methods – Relationship between pupal size and fat content

Work plan

1- Manipulate developmental conditions of *D. melanogaster* larvae to produce variation in size and fat content

2- Test the relationship between pupal size and fat content

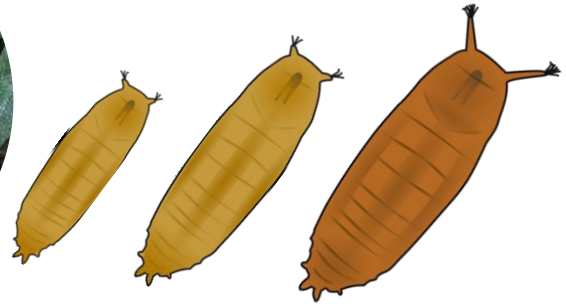
a - In laboratory-reared *D. melanogaster*

b - In field-collected *Drosophila sp.*

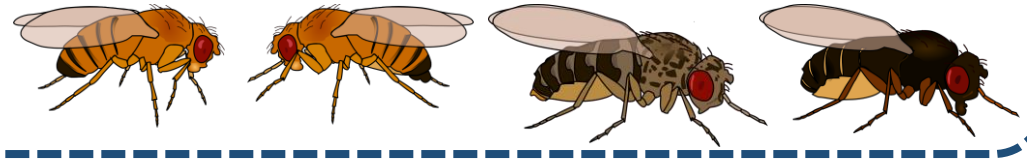
- Field-collected *Drosophila sp.*

Banana baits

Drosophila sp.

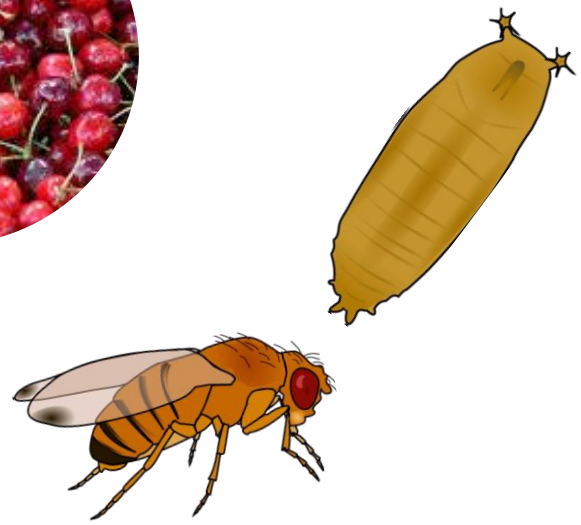


D. melanogaster, *D. simulans*, *D. hydei* and *D. subobscura*



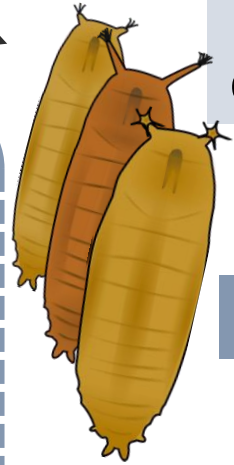
Infested cherries

Drosophila suzukii



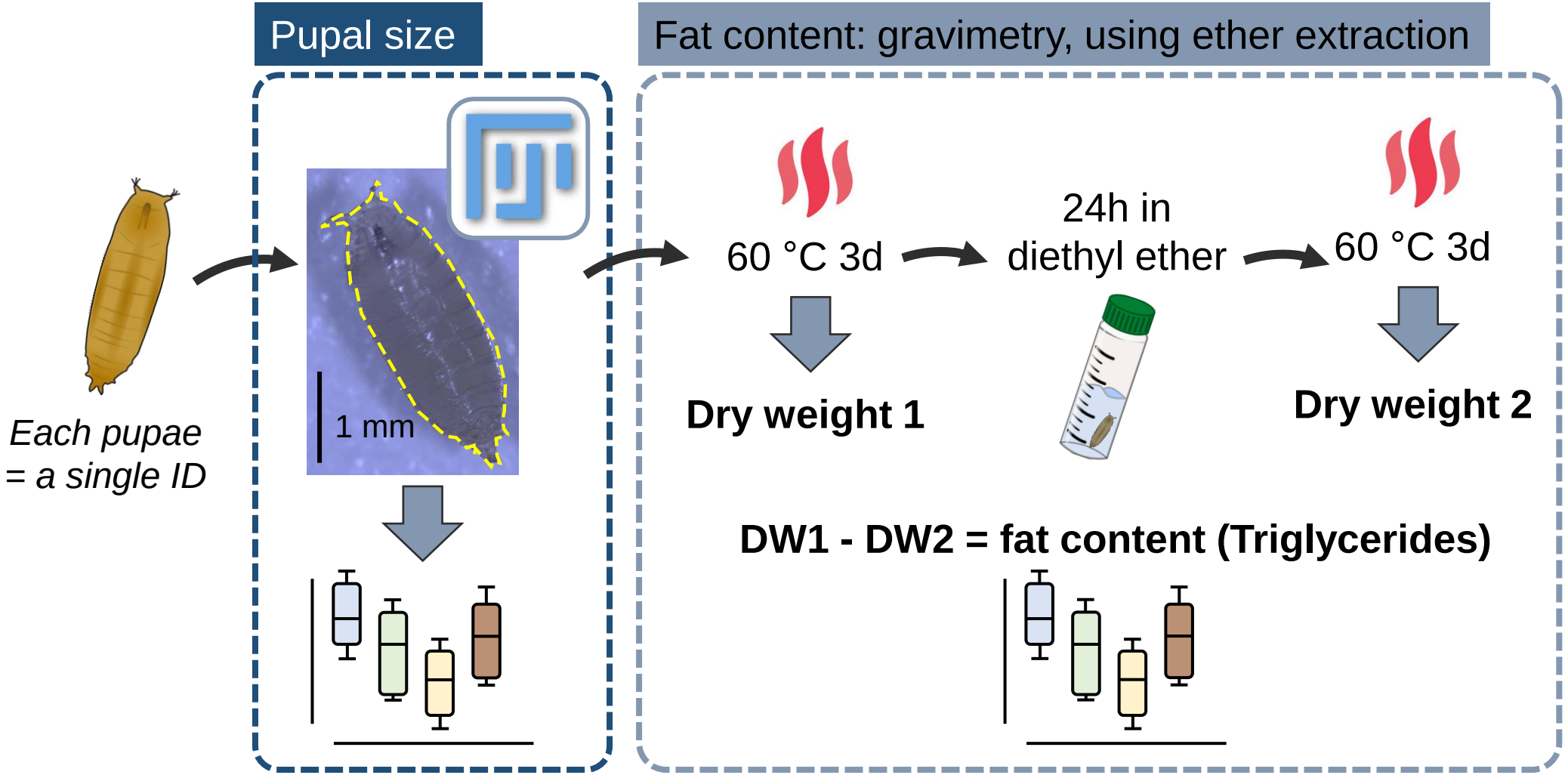
Fat content

Size



Methods – Relationship between pupal size and fat content

Size and fat content measurements

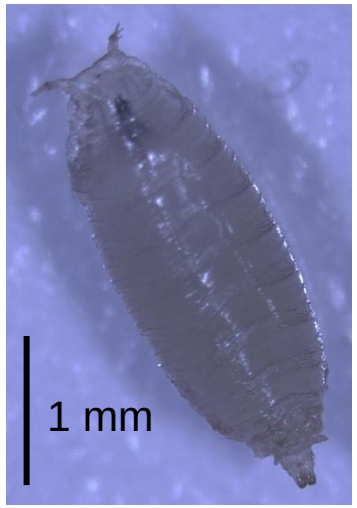


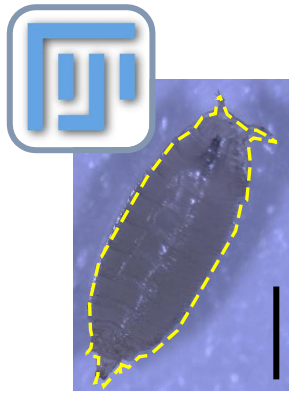
Results – Effects of developmental conditions

Laboratory-reared *D. melanogaster*

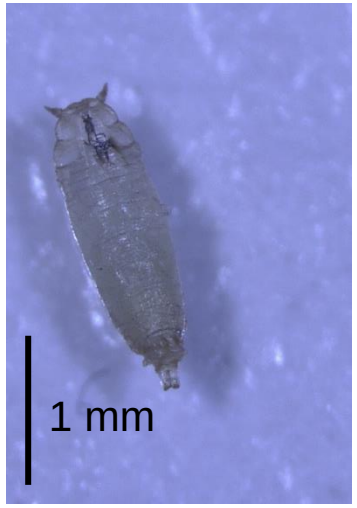
Pupal size **N = 375**

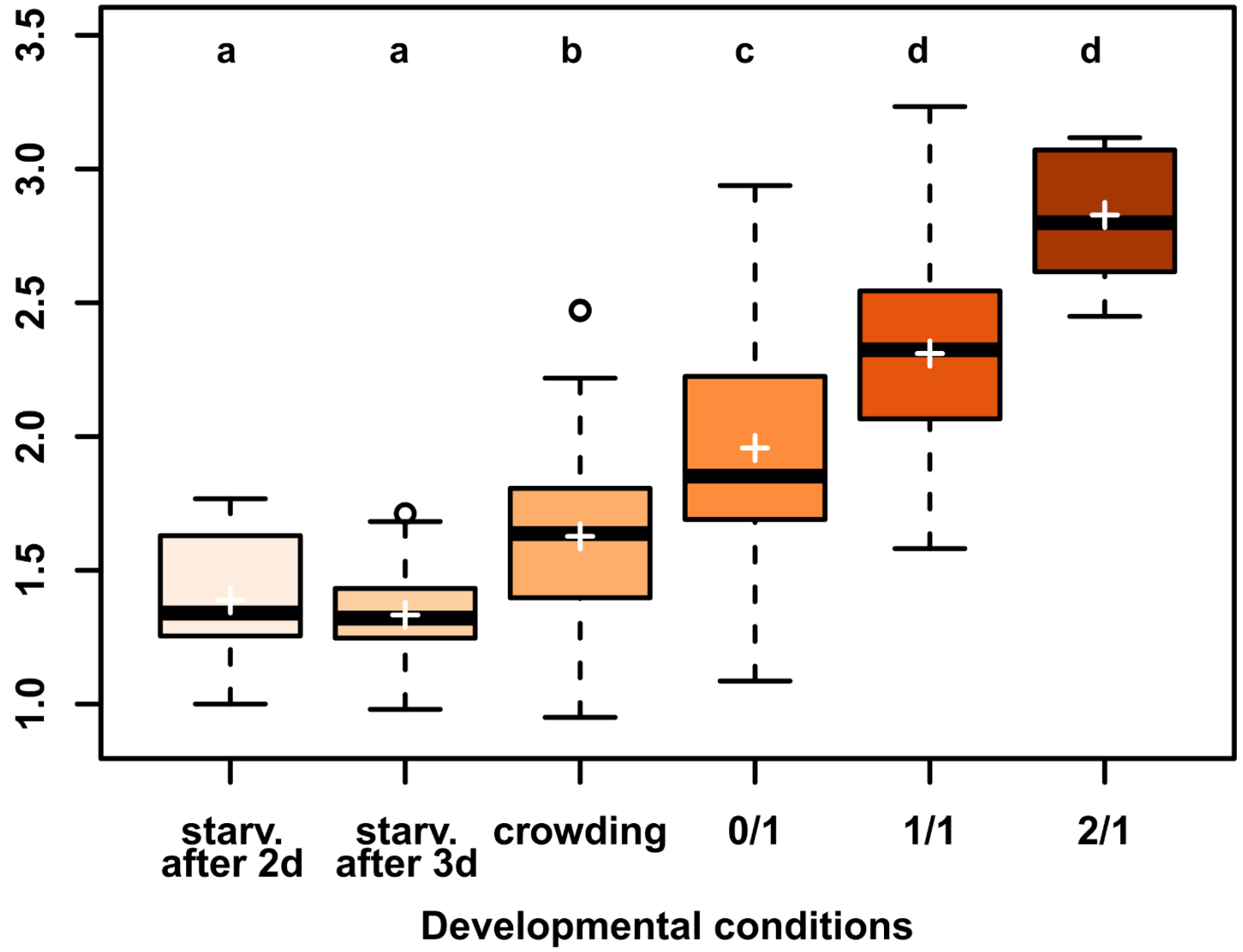
2/1





Starvation after 3d





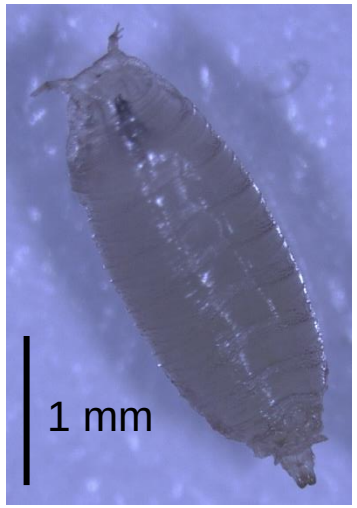
- GLMM, χ^2 = 665.43, df = 5, **p value < 0.001**
- Groups with different letters are significantly different (**p value < 0.05**; based on estimated marginal means comparisons)

Results – Effects of developmental conditions

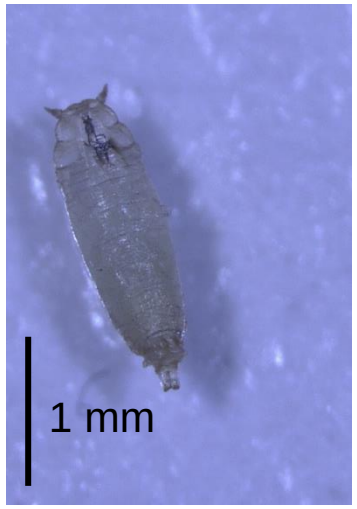
Laboratory-reared *D. melanogaster*

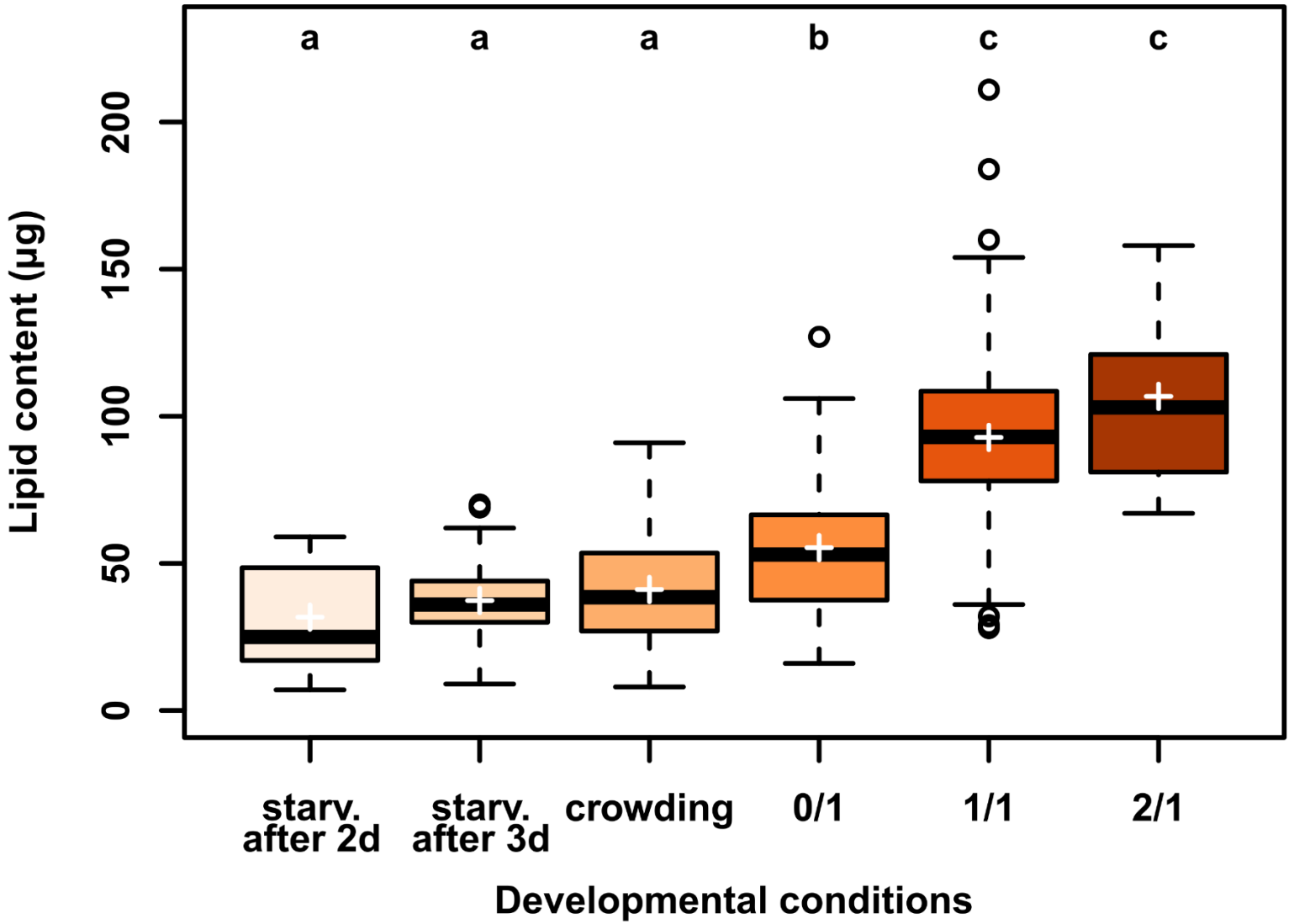
Pupal fat content **N = 375**

2/1



Starvation after 3d

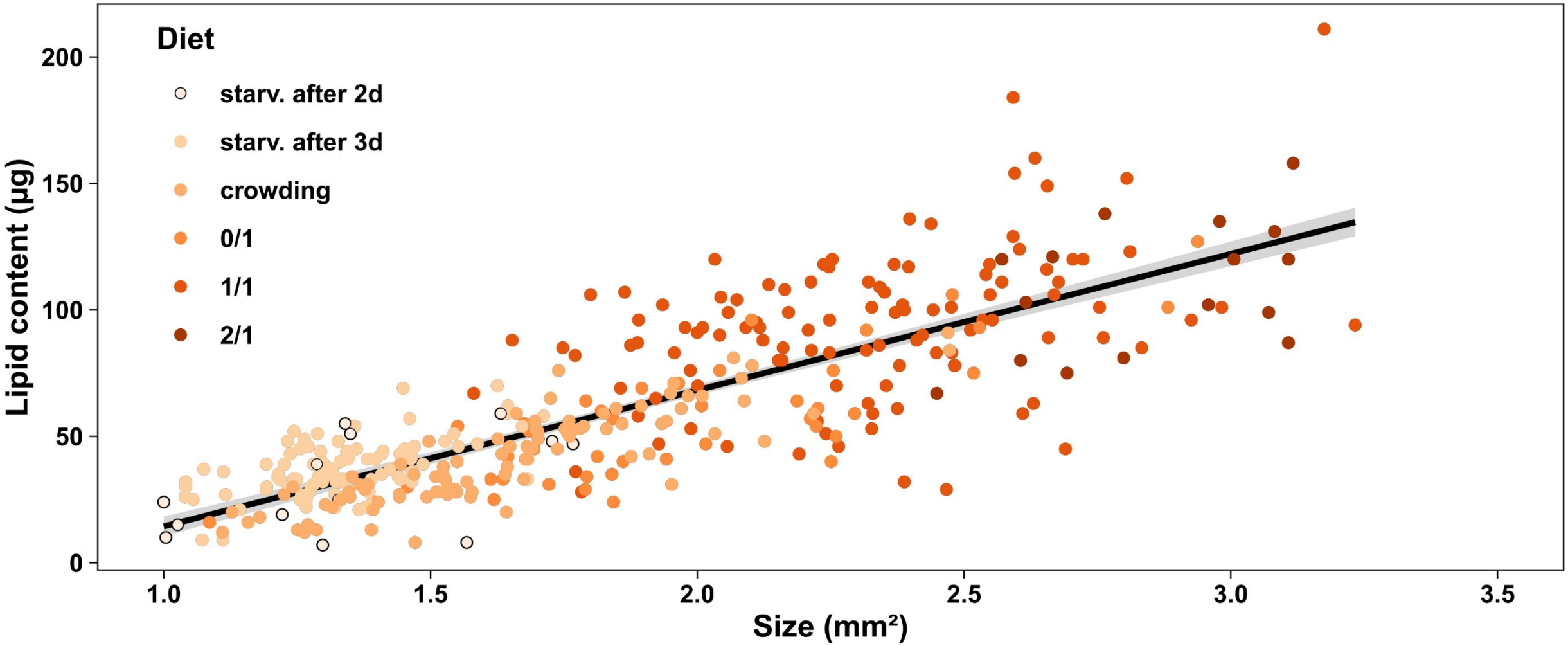




- GLMM, χ^2 = 412.92, df = 5, **p value < 0.001**
- Groups with different letters are significantly different (**p value < 0.05**; based on estimated marginal means comparisons)

Results – Correlation between pupal size and fat content

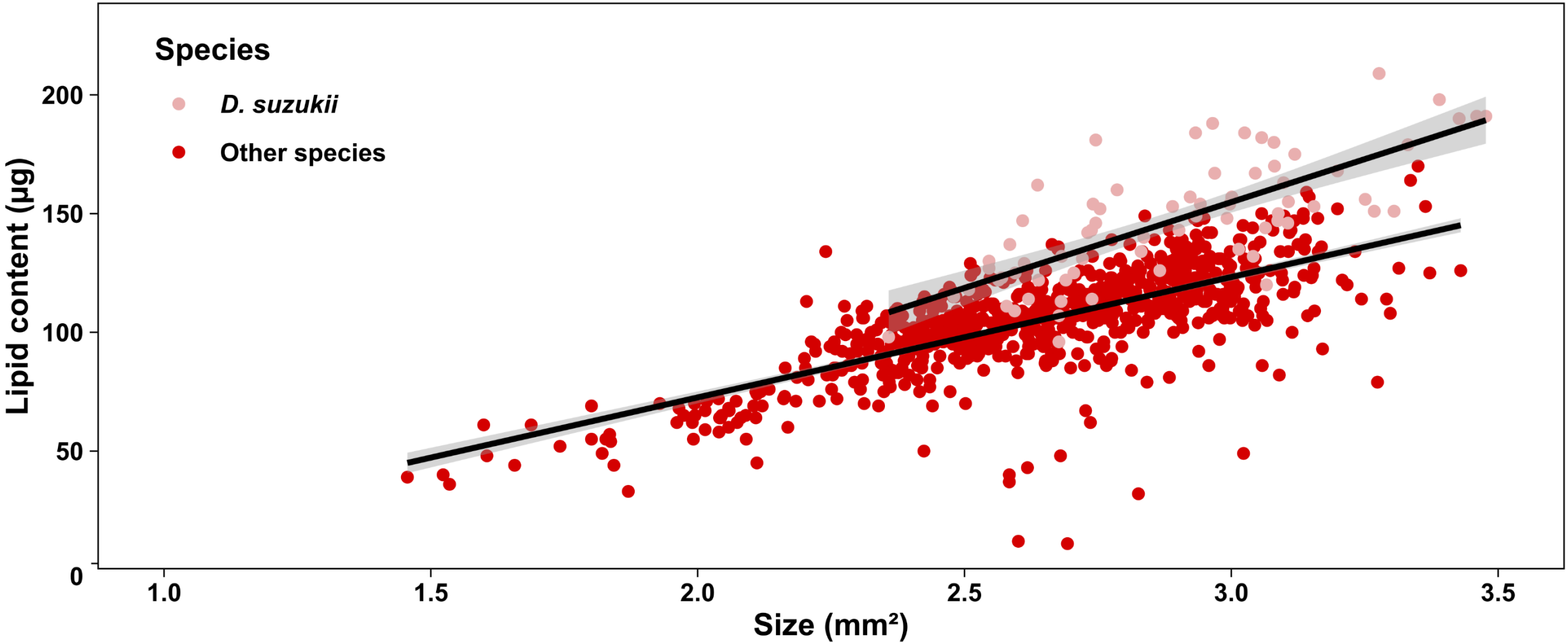
Laboratory-reared *D. melanogaster*



LMM, χ^2 = 190.86, df = 1, **p value < 0.001**, **marginal R^2 = 0.57, conditional R^2 = 0.85**, **N = 375**
(Size x Diet interaction : χ^2 = 2.47, df = 5, **p value = 0.78**)

Results – Correlation between pupal size and fat content

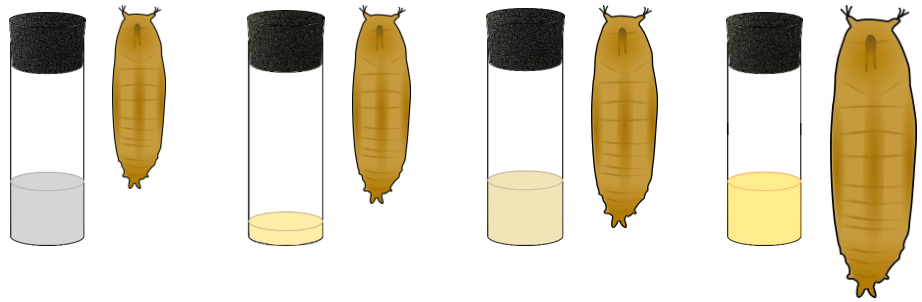
Field-caught *Drosophila sp.*



LMM, χ^2 = 688.08, df = 1, **p value < 0.001**, **marginal R^2 = 0.47, conditional R^2 = 0.78**, **N = 810**
(Size x Species interaction : χ^2 = 4.13, df = 1, **p value < 0.05**)

Discussion / conclusions

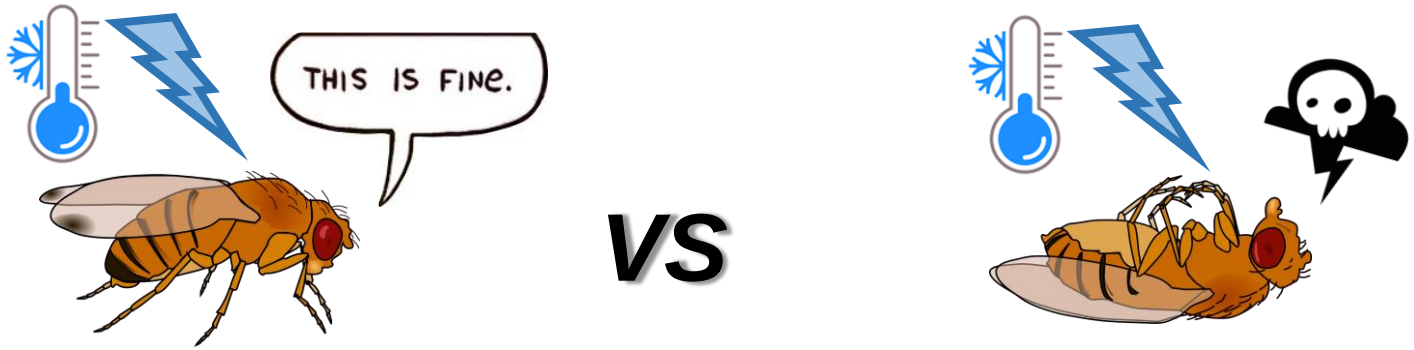
- Nutritional conditions largely affect size and fat content of *D. melanogaster* pupae
Starvation, low sugar, crowding… → In accordance with literature



(Borsh & Ho, 2001, J Insect Physiol; Henry et al, 2020, Comp Biochem Physiol A; …)

- Interestingly, *D. suzukii* contained more fat than other species.

→ Linked with cold tolerance?



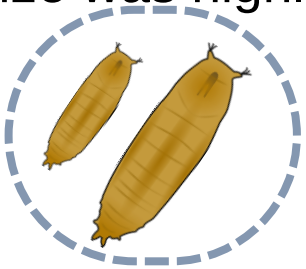
D. suzukii *D. melanogaster*

(Colinet & Hoffmann, 2012, Functional Ecol; Enriquez & Colinet, 2017, PeerJ)

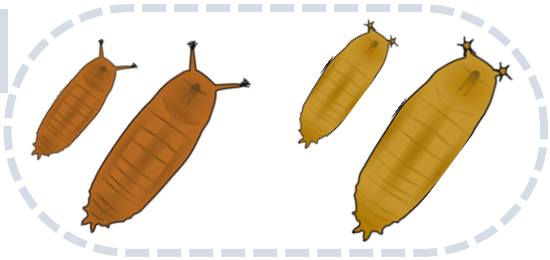
Discussion / conclusions

- As expected, pupal size was highly correlated with fat content

In laboratory-controlled conditions

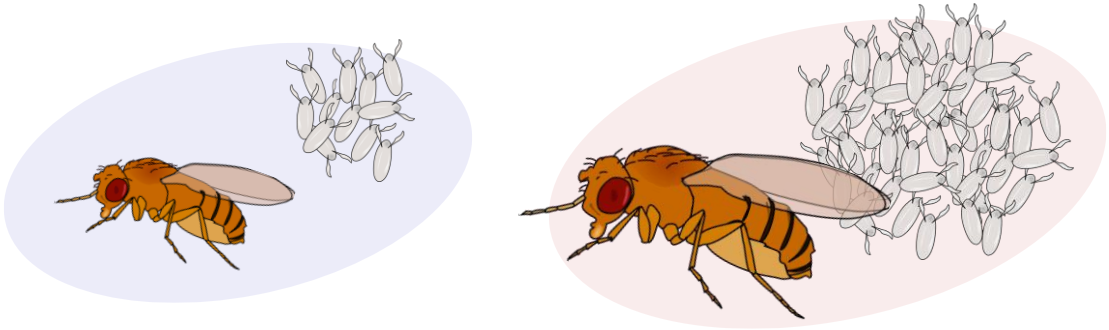


In wild individuals

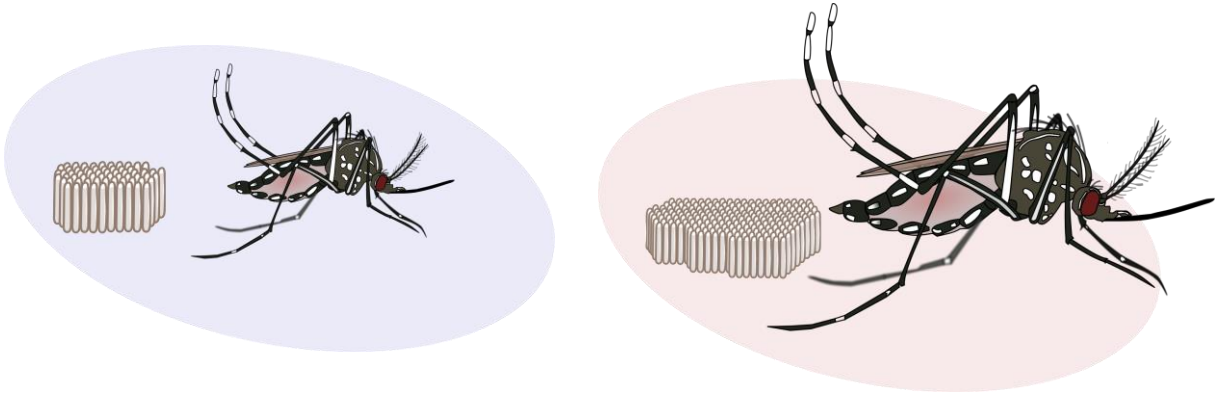


Of ecological relevance: larger individuals have higher fat reserves → influence other traits

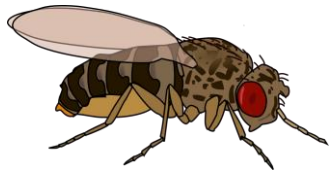
1,3 fold increase in size → 3 fold increase in fecundity (Tu & Tatar, 2003, Aging Cell)



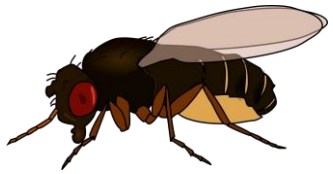
In *Aedes aegypti* : 1,4 fold increase in size → 3 to 6 fold increase in fecundity (Lounibos et al, 2002, J Vector Ecol)

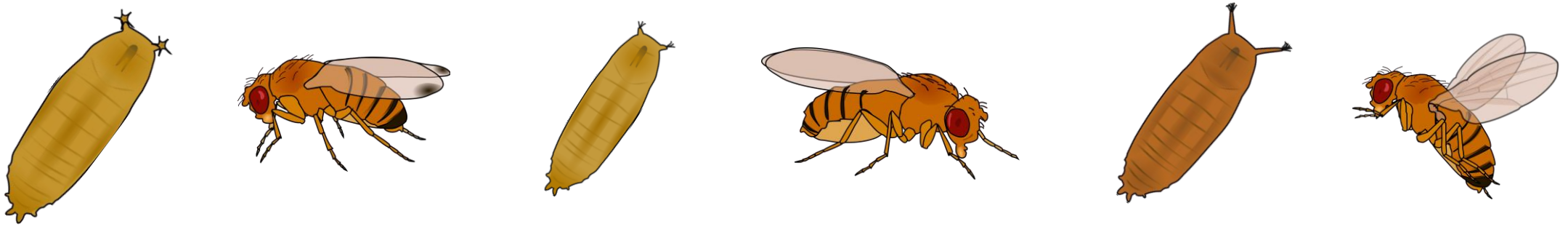


Pupal size represent a reliable, and relevant proxy of fat content, and therefore **Fitness**



Thanks for your attention !





Special thanks to:

- Organizing committee
- Bertanne Visser
- Caroline Nieberding
- Victoria Lievens
- And all the Entomology team in Gembloux

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