



Introduction

Male sexual ornaments are used to enhance mating success and are developmentally costly. For example, in *Themira superba* (Diptera: Sepsidae) the males take longer to complete development (~20hrs) and are nevertheless smaller than females.

Operational Sex Ratio (OSR) refers to the number of receptive females relative to the number of mature males. When a female-biased OSR evolves, males have more mating opportunities, mate competition is lowered for males, and therefore male sexual ornaments may be reduced.

Female-biased OSR occur frequently in arthropod species when they get infected with *Wolbachia* bacteria (~76% of all arthropod species). This bacteria selectively kills male offspring.

In order to study the effect of a female-biased OSR on the evolution of sexual ornaments, we conducted an artificial selection experiment with *T. superba*.

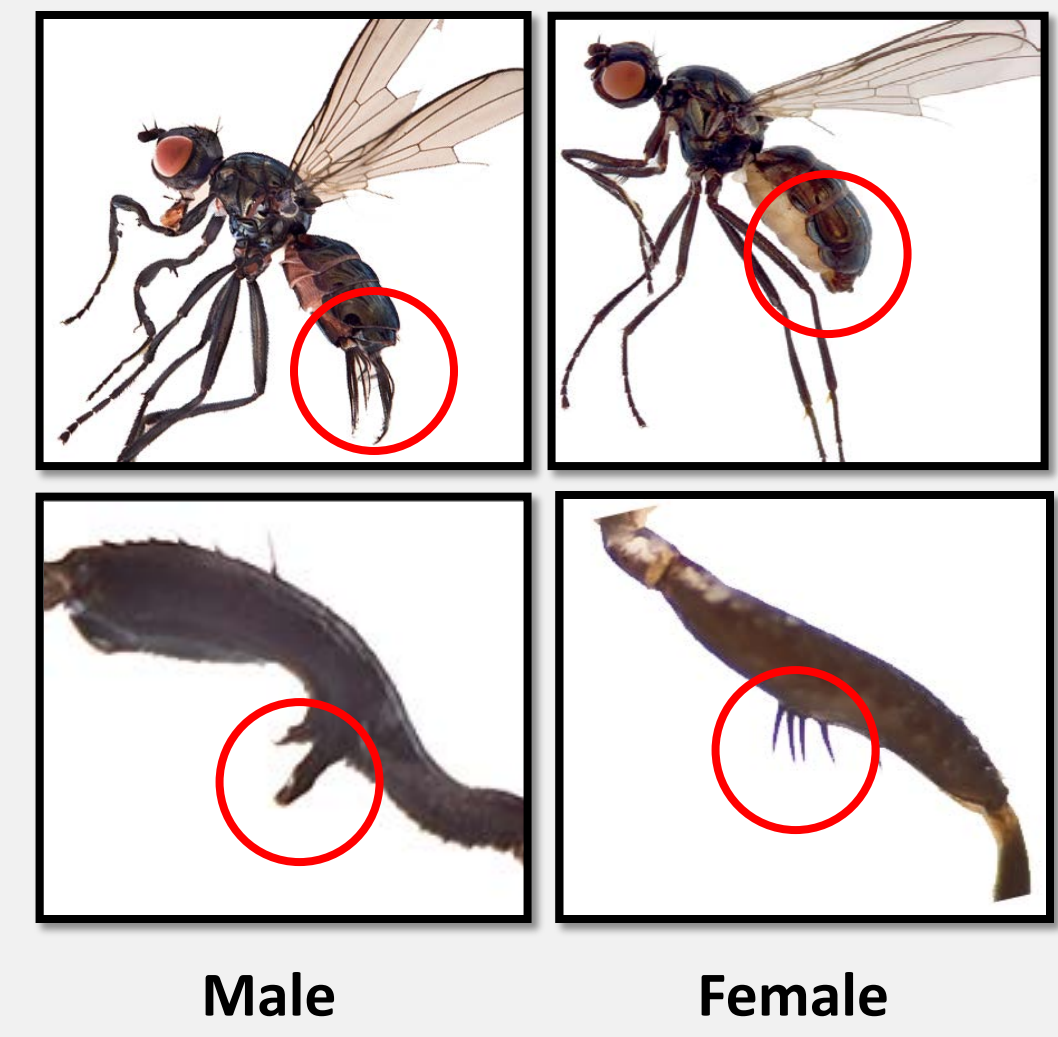


Figure 1: Dimorphic traits of *T. superba*

Objectives

- To determine how a prolonged female-skewed OSR will affect **sexual ornaments** that are **developmentally costly**
- To examine how a female-skewed OSR will affect **mating behavior**

Experimental Setup

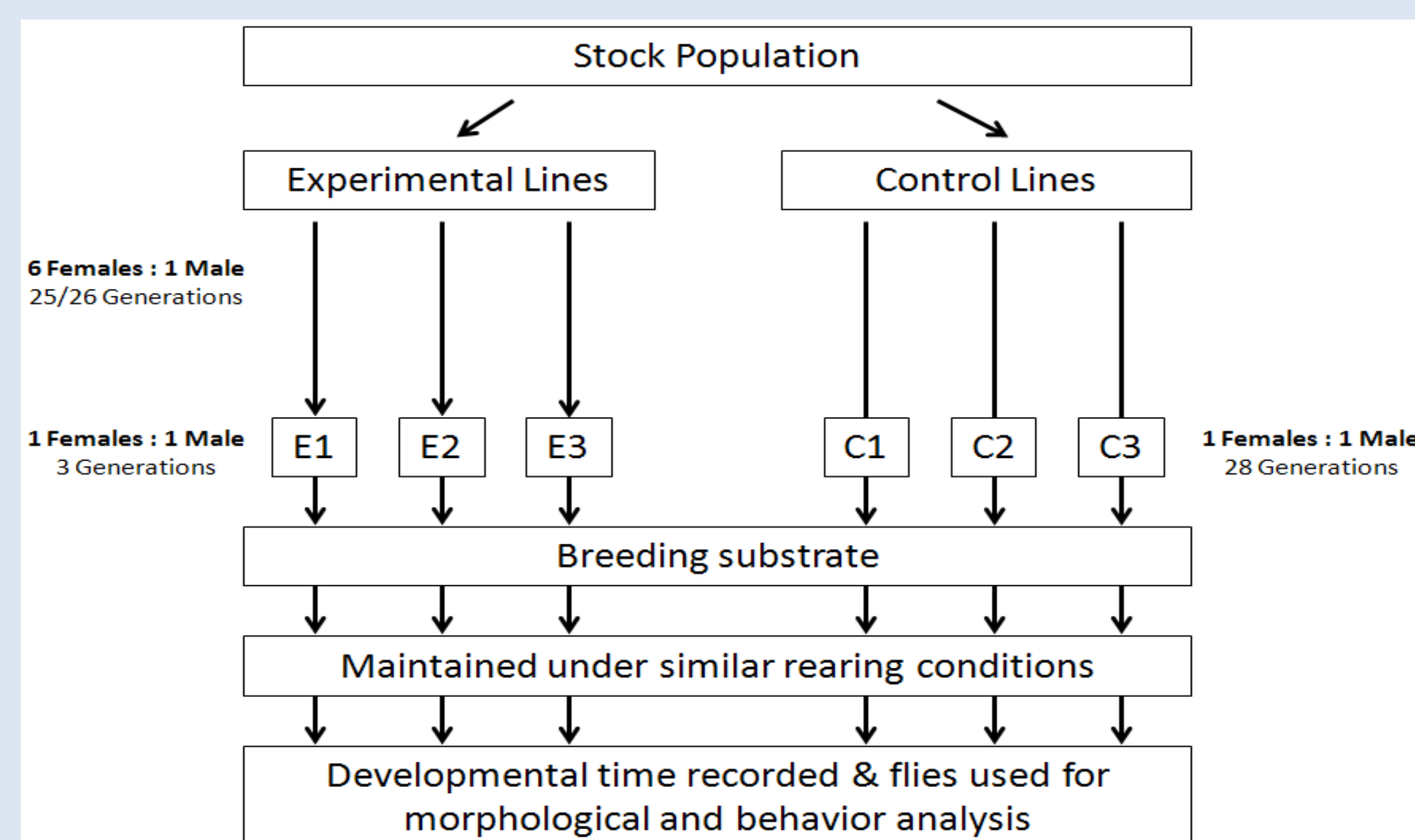


Figure 2: Flowchart of experimental setup

The starting population consisted of flies from a Belgian and a German population in order to increase genetic diversity

The generation time is ~16 days. 4 days worth of emergence were sexed and subsequently used to setup the next generation.

Experimental lines were under relaxed selection for at least 2 generations.

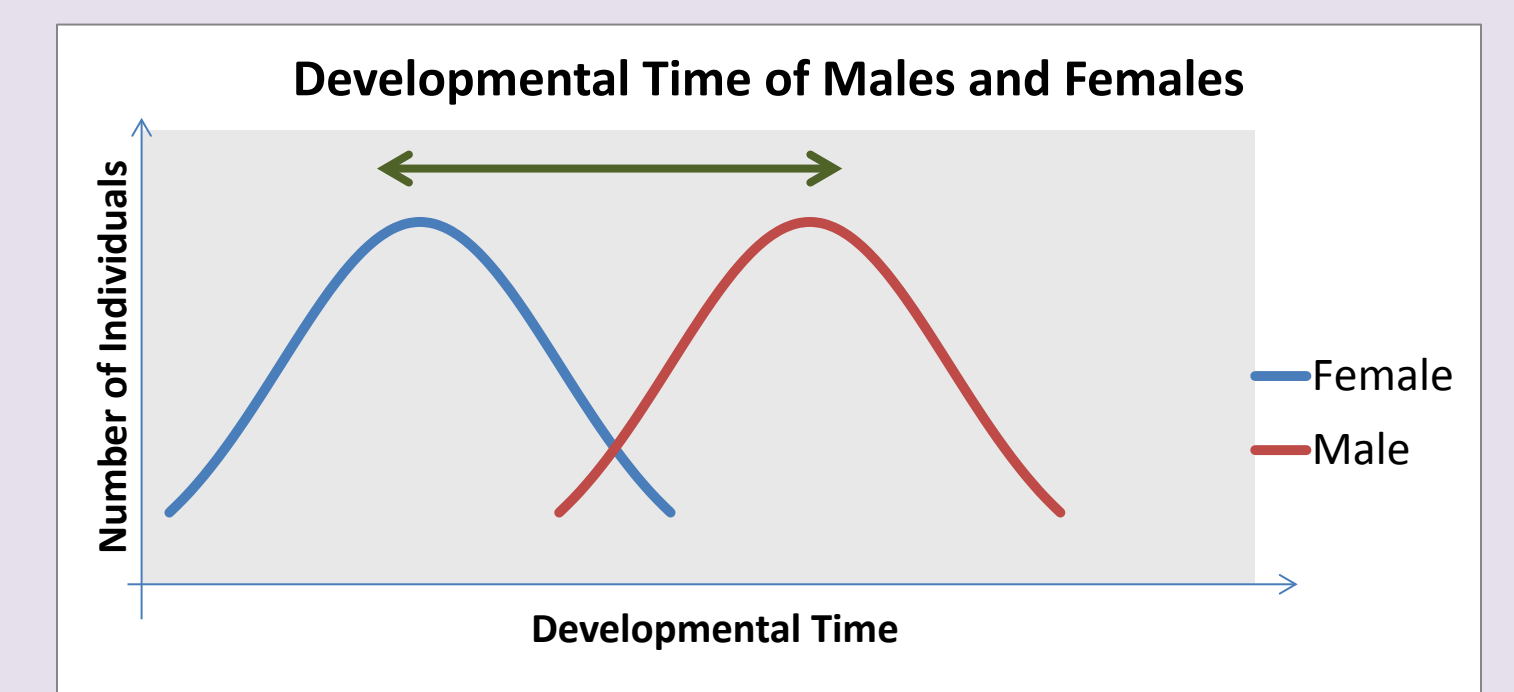
2) How do smaller sexual ornaments affect body size or developmental time?: Body size increases

Materials and Methods

- Body size of the 15 flies used for sexual ornaments measurement were quantified.
- Developmental time of every flies was quantified by recording the time at which they emerged



Figure 5: Male body size



Graph 1: Representation of developmental time of male and female flies.

Hypothesis

Less ornamental males would redirect resources to non-sexual traits or require fewer resources during development; i.e., they should either emerge (1) larger, (2) earlier, (3) or earlier and larger.

Finding

- Male body size increased significantly (**p-value = 0.00318**)
- There is no effect on developmental time

Potential trade-off between sexual and non-sexual traits

1) Sexual ornaments: Males reduce size and complexity of sexual ornaments

Materials and Methods

15 males were randomly selected from each line. Sexual ornaments were dissected and quantified.



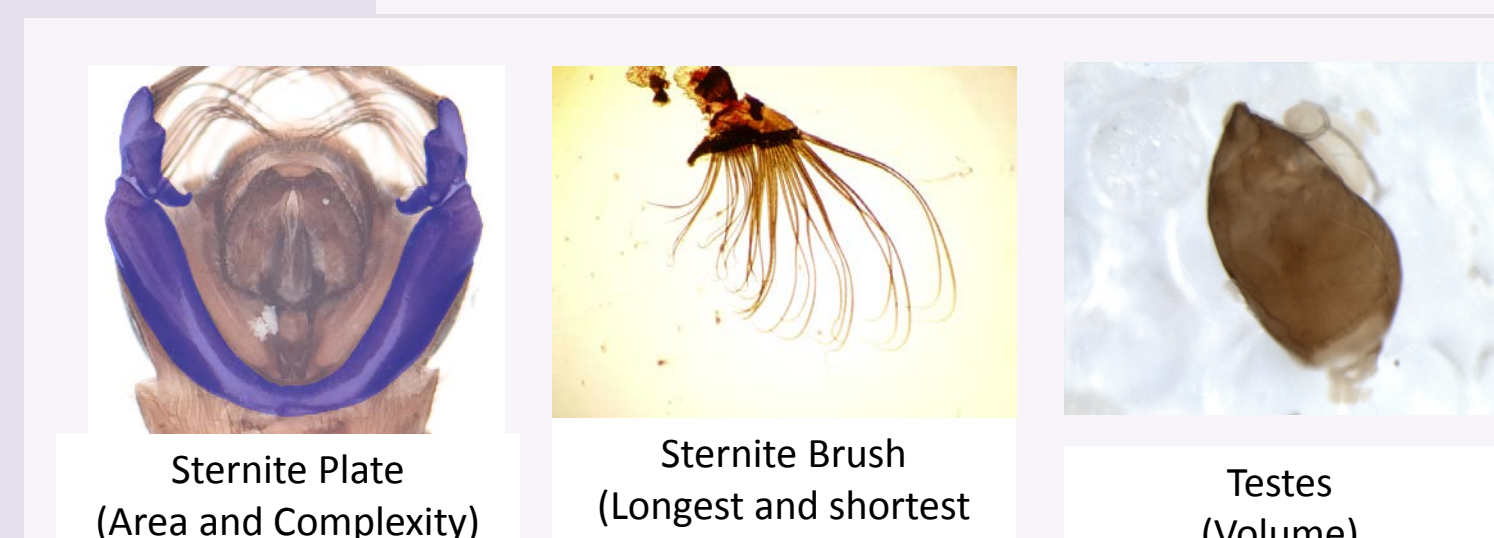
Foreleg (Area and Complexity) Clasper (Area and Complexity)

Hypothesis

Reduced investment in size and complexity of sexual ornaments

Finding

Hypothesis confirmed with PCA and Nested ANOVA



Sternite Plate (Area and Complexity) Sternite Brush (Longest and shortest length) Testes (Volume)

Figure 3: Male sexual traits measured

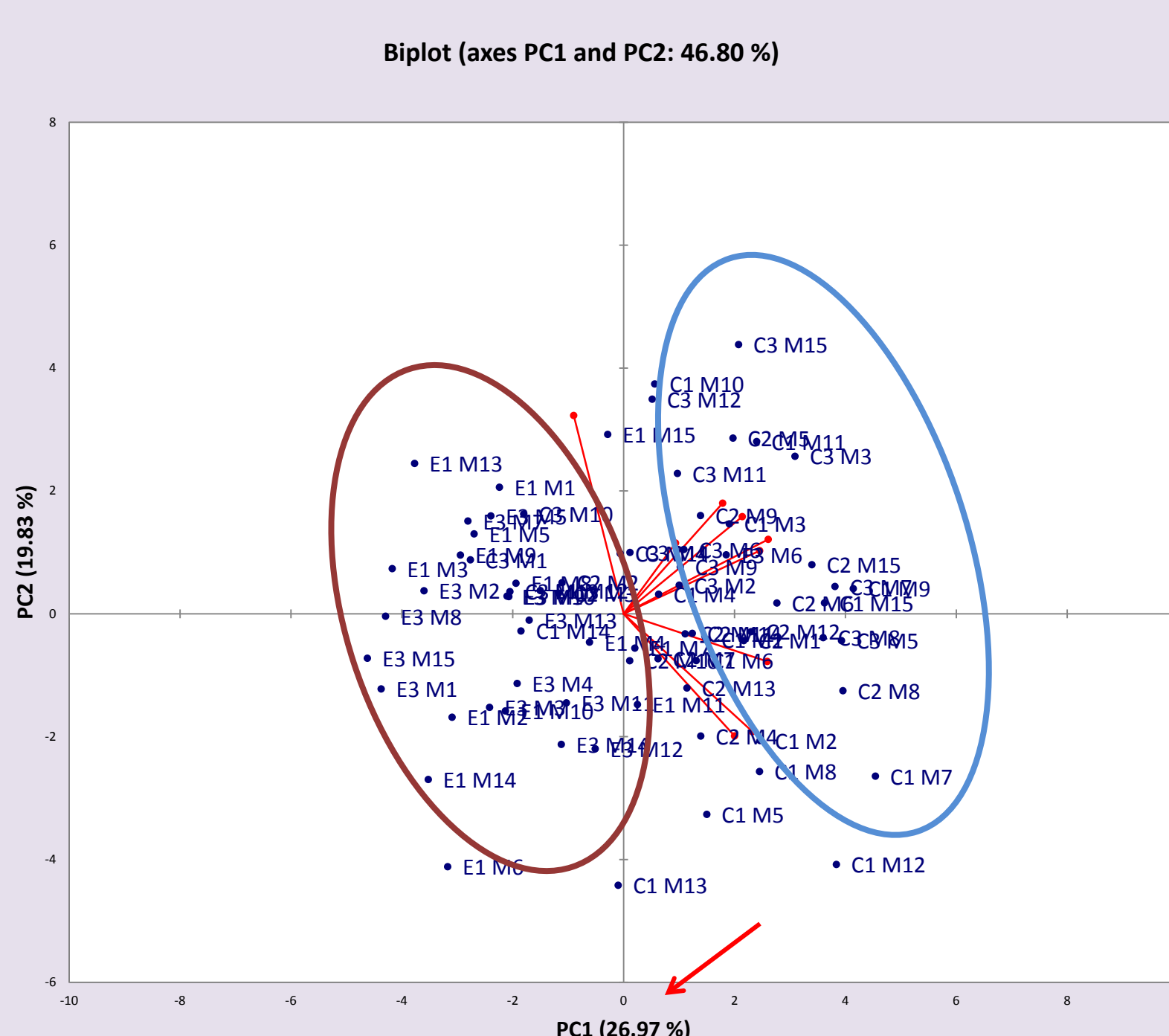


Figure 4: PCA biplots of sexual traits

Nested ANOVA shows that Experimental and Control differ significantly based on PC 1 scores (**p-value = 0.00212**)

Sexual Traits	PC1	Control Mean	Experimental Mean
Foreleg area	0.584	96891.22758	92480.41128
Sternite area	0.651	329058.2588	313215.9452
Clasper area	0.617	128828.5815	122006.9765
Long brush	0.656	1138.912492	1097.369519
Short brush	0.449	409.0303457	389.2721111
Testes	0.503	0.095773345	0.087981128
Sternite complexity	0.236	0.174763297	0.172643604
Foreleg complexity	0.226	0.124661787	0.127245288
Clasper complexity	0.538	0.365973454	0.339534684

Table 1: Factor loadings of PC1. Bold indicates significant contribution. Red indicate higher mean

3) Mating behavior: Copulation duration increases

Materials and Methods

15 virgin males and females from each line were randomly selected for 1:1 mating trials.

Hypothesis

- Females become less choosy as a result of the scarcity of males => higher mating success rate.
- Less effort from males to secure mating success.
- Copulation duration would decrease



Figure 6: Copulation of *T. superba*

Findings

- No difference in mating success
- No difference in male effort
- But copulation duration increases significantly (**p-value = 0.004653**)

Are females unwilling to let go so that they can secure more sperm?

Qualitative observations showed that Experimental males were having more difficulties in post-copulatory separation

Conclusion: The artificial selection experiment provides evidence to show that a female-biased OSR can cause reduction in the magnitude of sexual ornaments and potentially changing mating behavior of Sepsids. This experiment also suggests that a bacterium can strongly affect the evolution of an eukaryote.