Age dependent sexual maturation of *Vespa velutina* males : an anatomic approach.

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Introduction

Vespa velutina, an invasive predator of bees

The Yellow legged hornet *Vespa velutina var nigrithorax* is a Chinese invasive predator of bees accidently introduced in France 11 years ago in the Lot et Garonne (France). The biology and behavior of this alien species is poorly understood, both in its native area and in Europe. In order to control this pest, studying traits related to reproductive success is needed. The present work focus on the male reproductive physiology.

Biology of *Vespa velutina*

V. velutina has an annual development cycle that begins in February, the colony being initiated by an overwintering fertilized queen. The males are produced by non fertilized eggs, mostly laid by the queen in summer and autumn, and more rarely by workers with maturated ovarian from a queenless colony (2,3).

Questions :

1- Do anatomic and physiological changes in *Vespa velutina* males occur during its adult life ?

2- Is head size (as a body size indicator) correlated to spermatozoa production ?

Objective of this study

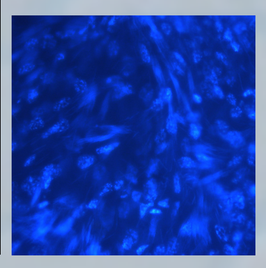
4 nests were collected in the area of Bordeaux in November 2014, and their combs were maintained in climatic chambers (23 ± 1 °C, LD : 12/12). We dissected 72 males of different ages, and described 3 traits : testes size, spermatogenesis, and number of spermatozoa available in the seminal vesicles.

Material and methods

1– Dissection and morphology

Measurement of the testis area using an image processing program, ImageJ1® , and of the head size using a digital calliper.

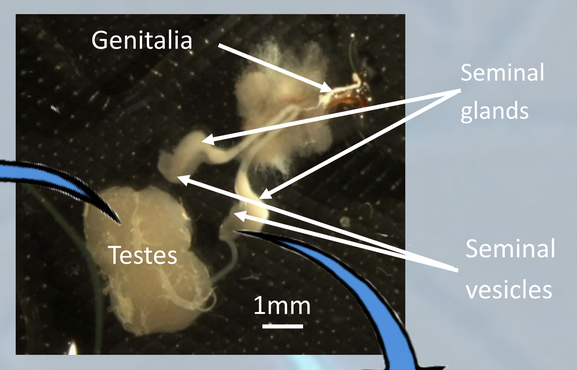
2- Spermatogenesis in the testes



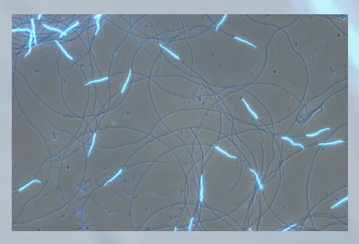
Observation of the testes follicles

Differentiation stages of the spermatids.

A: few spermatids with round nuclei. B: more than 16 round nuclei. C : the nuclei becomes tapered. D : the nuclei dissociates on one side. E : the new spermatozoa are free.



3– Counting spermatozoa in the seminal vesicles



For each male, we estimated the number of spermatozoa in each seminal vesicle (n1+n2).

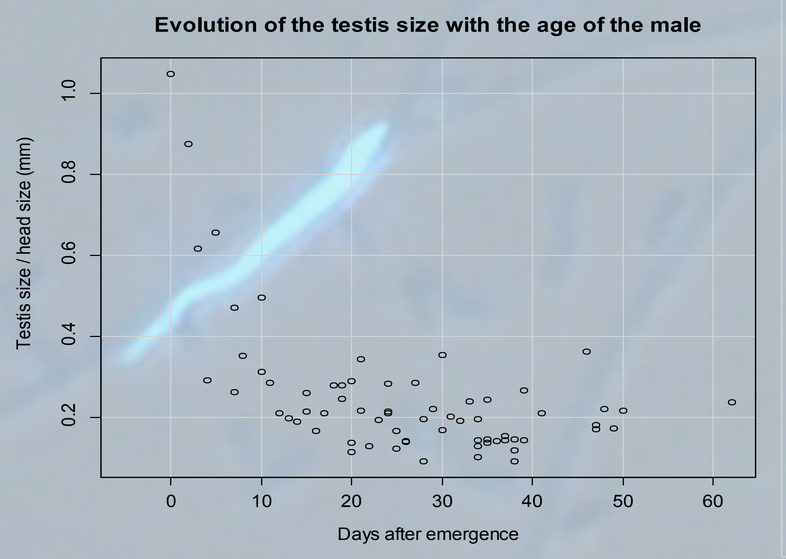
$$N = (a^* (R1 \times R2 \times \pi) / f) + (b^* (R3 \times R4 \times \pi) / f)$$

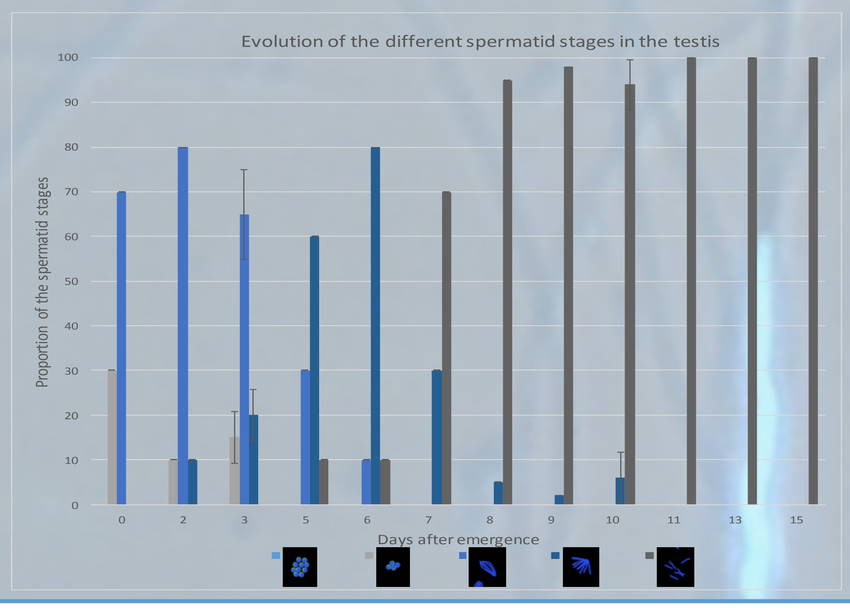
a , b : total number of spermatozoa counted in 5 microscope fields in each seminal vesicle

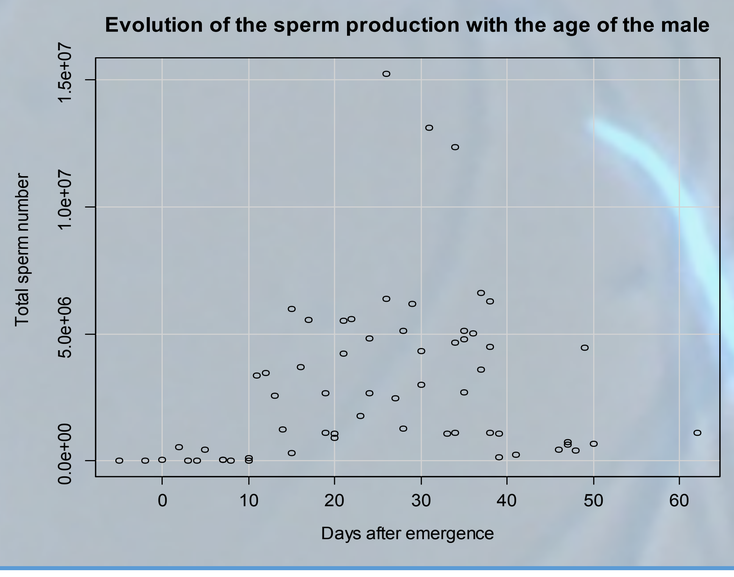
(R1 , R2) ; (R3, R4) : the 2 radii of the drop where each seminal vesicle was spread.

f : size of 5 microscope fields.

Results







Conclusions

. Males are able to transfer sperm 10 days after emergence.
. The spermatogenesis ended 11 days after emergence, and the testes degenerated until this date.
. The spermatogenesis is synchronic, with only one wave of sperm production.
. After 10 days, up to 15.10^6 spermatozoa can be found in the males seminal vesicles.
. No correlation was found between the male's head size and its spermatozoa production, or the size of its testes after 11 days.
. Given those results, the possibility of multiple mating in males is not likely to occur.

Bibliography

(1) Monceau, Bonnard, Thiéry, *"Vespa velutina* : a new invasive predator of honeybees in Europe", *Journal of Pest Science*, (2013) (a).

(2) Takahashi J., Akimoto S., Martin S. J., Tamukae M., Hasegawa E. , "Mating Structure and Male Production in the Giant Hornet *Vespa mandarinia* (Hymenoptera: Vespidae)." *Applied Entomology and Zoology* 39, no. 2 (2004): 343–49.

(3) Spiewok S., Schmolz E., Ruther J. ,"Mating System of the European Hornet *Vespa Crabro*: Male Seeking Strategies and Evidence for the Involvement of a Sex Pheromone." *Journal of Chemical Ecology* 32, no. 12 (December 4, 2006): 2777–88.

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