





**Designação do projeto** Communication during courtship: the role of ovipositor extrusion

Código do projeto | LISBOA-01-0145-FEDER-30105

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Objetivo principal | Reforçar a investigação, o desenvolvimento tecnológico e a inovação

Região de intervenção | Lisboa

Entidade beneficiária | FUNDAÇÃO D. ANNA DE SOMMER CHAMPALIMAUD E DR. CARLOS MONTEZ CHAMPALIMAUD

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## Objetivo |

In fruit flies, as with many animal species, the male decides whether to court, and the female decides whether or not to mate with the courting male. We aimed at understanding the communication between male and female during courtship. In particular how song and ovipositor extrusion contribute to the progression of courtship.

## Atividades |

We assessed behaviourally how ovipositor extrusion influences courtship behaviour. Using optogenetics we activated the ovipositor extrusion neurons in the female, i.e. induced ovipositor extrusion, while the male courts her. We did the reverse manipulation of suppressing ovipositor extrusion during courtship. Finally, we analysed the behavioural consequences of the manipulations; We investigated which male sensory stimuli are relevant for female ovipositor extrusion; We explored the female response to the male song.

## Resultados atingidos e em progresso |

Communication between male and female fruit flies during courtship is essential for successful mating, but, as with many other species, it is the female who decides whether to mate. Here, we show a novel role for ovipositor extrusion in promoting male copulation attempts in virgin and mated females and signalling acceptance in virgins. We first show that ovipositor extrusion is only displayed by sexually mature females, exclusively during courtship and in response to the male song. We identified a pair of descending neurons that controls ovipositor extrusion in mated females. Genetic silencing of the descending neurons shows that ovipositor extrusion stimulates the male to attempt copulation. A detailed behavioural analysis revealed that during courtship, the male repeatedly licks the female genitalia, independently of ovipositor extrusion, and that licking an extruded ovipositor prompts a copulation attempt. However, if the ovipositor is not subsequently retracted, copulation is prevented, as it happens with mated females. In this study, we reveal a dual function of the ovipositor: while its extrusion is necessary for initiating copulation by the male, its retraction signals female acceptance. We thus uncover the significance of the communication between male and female that initiates the transition from courtship to copulation.

Persuasion is a crucial component of the courtship ritual needed to overcome contact aversion. In fruit flies, it is well established that the male courtship song prompts receptivity in female flies, in part by causing sexually mature females to slow down and pause, allowing copulation. Whether the above receptivity behaviours require the suppression of contact avoidance or escape remains unknown. Here we show, through genetic manipulation of neurons we identified as required for female receptivity, that male song induces avoidance/escape responses that are suppressed in wild type flies. First, we show that silencing 70A09 neurons leads to an increase in escape, as females increase their walking speed during courtship together with an increase in jumping and a reduction in pausing. The increase in escape response is specific to courtship, as escape to a looming threat is not intensified. Activation of 70A09 neurons leads to pausing, confirming the role of these neurons in escape modulation. Finally, we show that the escape displays by the female result from the presence of a courting male and more specifically from the song produced by a courting male. Our results suggest that courtship song has a dual role, promoting both escape and pause in females and that escape is suppressed by the activity of 70A09 neurons, allowing mating to occur.

The results summarized above were published in international peer reviewed journals: Mezzera C, Brotas M, Gaspar M, Pavlou HJ, Goodwin SF, Vasconcelos ML. 2020. Ovipositor extrusion promotes the transition from courtship to copulation and signals female acceptance in Drosophila melanogaster. Curr. Biol. 30:3736-3748. Arez E, Mezzera C, Neto-Silva RM, Aranha MM, Dias S, Moita MA, Vasconcelos ML. 2021. Male courtship song drives escape responses that are suppressed for mating. Sci Rep. 11(1):9227.

Additionally, the results were disseminated in:

four international and two national oral presentations, a Master thesis and a Radio interview.