Comment

Does surgical manipulation of *Drosophila* intromittent organs affect insemination success?

1. INTRODUCTION

Surgical ablation is a powerful approach for verifying the function of focal animal body parts. Polak & Rashed [1] (hereafter P & R) have developed a new micron-scale laser surgery technique and applied it to study the function of the claw-like genital spines (spines of ventral cercal lobes; highlighted in blue in figure 1) of *Drosophila bipectinata* Duda (Diptera, Drosophilidae). They successfully ablated the genital spines either partly or completely, and studied the effects of these manipulations on a suite of reproductive traits. To our knowledge, this is the first successful case of the surgical manipulation of a micron-scale genital trait in any insect. Based on subsequent observations of treated males, P & R concluded that the excision of the spines sharply reduced mating success compared with controls. However, when copulation occurred, fertilization rates, in either competitive or non-competitive contexts, were not statistically different between the treated and control males. P & R concluded that the genital spines mainly function to mechanically couple the male and female genitalia, and that they may be evolving in response to pre-insemination sexual selection.

For *D. bipectinata* (and three related species), one the authors of this comment (Y.K.) has reported the occurrence of traumatic insemination: a pair of claw-like organs, termed basal processes of the aedeagus (highlighted in orange in figure 1), penetrate the female body wall near the genital orifice and transfer sperm into the genital tract through the wounds during copulation [2]. Below, we explain that P & R (pp. 1371–1372) incorrectly interpreted these basal processes described in Kamimura [2] to be the structures they manipulated with the laser. We clarify that the genital spines studied by P & R and the basal processes studied by Kamimura [2] are distinct structures with distinct functions. Recommendations are made to minimize ambiguity in future studies of insect genitalia.

2. DETAILED GENITAL ANATOMY OF MALE *DROSOPHILA*

As in other *Drosophila* species, the male genitalia of *D. bipectinata* are very complicated, consisting of the phallic parts (aedeagus, basal processes of the aedeagus, parameres and gonopods), most of which are inserted into the female genital tract during copulation, and the surrounding periphallal parts (epandrium, cerci, ventral cercal lobes, surstyli and ventral lobes; figure 1; see also [2]). In *D. bipectinata* (and the three other species of the

D. bipectinata species complex), the aedeagus, which functions as a sperm-transferring organ in other drosophilids, is much degenerated into a colourless, soft, membranous structure [2]. Instead, a pair of basal processes of the aedeagus is well developed as robust claw-like structures in this species (highlighted in orange in figure 1a,b; [2]). It is these claw-like structures that have been implicated in traumatic insemination in *D. bipectinata* and related species [2].

The scanning micrographs in fig. 1 of P & R indicate that they experimentally ablated a pair of prominent apical spines located between the surstyli and cerci (highlighted in blue in figure 1), and not the claw-like basal processes of the aedeagus studied by Kamimura [2] (figure 1). P & R correctly noted that these apical spines arise from the ventral cercal lobes, consistent with the terminological system adopted by dipterologists [3].

The apical spines of the ventral cercal lobes studied by P & R is a periphallal structure shared by most members of the *D. ananassae* species subgroup to which *D. bipectinata* belongs ([4]; termed secondary claspers in this reference), and it is evolving rapidly, as revealed by its diversification in size and shape among species. Using a freeze-fixation technique for mating pairs, Eberhard & Ramirez [5] studied the genital coupling of *D. malerkotliana* Parshad & Paika, which is characterized by a male genital structure essentially the same as that of *D. bipectinata*. Figs 6 and 9 of Eberhard & Ramirez [5] show that the ventral cercal lobes mesh with the outer surface of the female oviscapts (termed oviscape valves in this reference) during copulation. Thus, the apical spines of the ventral cercal lobes do not function as intromittent organs in the strict sense of inserting into the female genital tract, but can be considered as a pair of clasping devices to hold the female securely.

In articles on evolutionary or functional aspects of insect genitalia, the use of specialized taxonomic terms is often avoided in specifying the structures under consideration, and this is a possible cause of misinterpretation. For example, male drosophilids have multiple genital structures that seem to function as clasping organs (cerci, ventral lobes and surstyli; figure 1b). Use of the common term ‘claspers’ may be beneficial for general readers not familiar with the focal taxon, but it would also hinder intellectual exchanges among behavioural or evolutionary biologists, morphologists and taxonomists working on genital structures. To minimize ambiguity, it would be worthwhile to include both specialized and common terms in articles on insect genitalia, together

Figure 1. (a) A scanning electron micrograph and (b) a schematic drawing of male genitalia in *D. bipectinata*. Caudal views. Basal processes of the aedeagus (highlighted in orange) and the gonopods (highlighted in green) are phallic structures, while the other colour-coded parts are peripheral. In (b), the hypandrium and parameres are omitted for simplicity. Pubescence and most setae (excluding stout ones) are also omitted. The terminological system used by general dipterologists [3,8] is adopted. In (a), each part is highlighted in the same colours as in (b). Scale bar, 50 μ m.

with an indication of the terminological system adopted. This would be especially important for *Drosophila*, in which the genital terminology and the homologies of many genital structures are not settled (see table 2 of [6]).

3. CONCLUSION

In conclusion, P & R have experimentally tested the function of the spines on the ventral cercal lobes, which are distinct from the claw-like basal processes of the aedeagus

studied by Kamimura [2]. Moreover, the genital spines should not be considered intromittent *sensu stricto*, although these structures do mesh with female external genitalia, and only in this sense may they be thought of as 'intromittent'. The results of P & R. indicate that the spines may be evolving in response to sexual selection occurring prior to insemination, although other alternatives which are clearly important in other species, such as cryptic female choice [7], require further investigation. Application of the new excision technique developed by P & R to the phallic or intromittent organs remains as a challenge for the future. When not engaged in copulation, the phallic parts are usually located behind the surstyli (see fig. 1a of P & R). To manipulate them without harming the surrounding parts, we need to develop an additional, non-damaging technique, to expose the phallic organ of anaesthetized flies.

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