displacement in each cross of remated females, which range from 0.60 to 0.67 extending the evidence for sperm displacement in *D. bipectinata*.

**Keywords:** *Drosophila bipectinata*, female remating, postcopulatory sexual selection, sperm displacement.

When a female insect mates with multiple males their ejaculate may temporally overlap, generating intrasexual conflict between sexes over paternity\(^6\), which is an indirect consequence of female remating, selection on male traits that enhance competitive fertilization success\(^3, 5\) and selection on female traits that mediate cryptic female choice\(^6, 7\). These selective pressures collectively constitute postcopulatory sexual selection which generates variation in male and female behaviour\(^8\). Postcopulatory sexual selection includes both male–male competition (sperm competition) and female choice (cryptic female choice)\(^9\) and plays a profound role in population divergence\(^9, 10\).

Traditionally, sperm competition has been seen as an intra-sexual conflict with the female being an inert arena in which the conflict occurs\(^9\). In the reproductive tract, females exert choice (cryptic female choice) on the sperm and select the most compatible sperm. However, sperm of the males compete among themselves for fertilizing the eggs and the one which is superior wins the battle. Thus both males and females play a role in the selection process.

The existence and relevance of sperm competition in *Drosophila* has been a contentious issue in evolutionary genetics\(^10\). The phenomenon of sperm competition occurs in many insect species, particularly in *Drosophila* females because of: (i) ability of females to store sperm from different males\(^11-14\), (ii) the highly efficient use of stored sperm at fertilization\(^13, 15\), and (iii) the high probability of multiple mating\(^2\). After remating it has been observed that the sperm from the last (or second) male usually takes precedence over those of previous males and preferentially fertilizes subsequent eggs, a phenomenon known as sperm displacement or sperm precedence\(^16\).

The study of sperm competition has centred around the question of whether females tend to remate only after most of the stored sperm has been utilized, i.e. sperm dependence of remating\(^14\), or whether remating occurs relatively rapidly and before the first male sperm has been substantially depleted\(^2\). Most studies of postcopulatory sexual selection have focused on the pattern of sperm precedence, such as the proportion of progeny sired by the second male in a double mating trial (P2). The P2 value varies from 0 to 1. A P2 value of 0.5 is usually taken as evidence that the sperm of the two males are equally mixed in store. Whereas P2 value of 0 or 1 may indicate that the sperm of the first or second males has gained complete precedence over that of other males, or that sperm from the first or second male has become depleted or lost\(^9\).

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**Studies on remating behaviour in the *Drosophila bipectinata* species complex: evidence for sperm displacement**

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In *Drosophila bipectinata* female remating with respect to productivity and sperm displacement was studied by employing two mutant strains and a wild-type strain. The comparison of productivity between once-mated (control) and remated females revealed that the productivity of remated females is significantly higher than that of once-mated females in all the crosses showing increased productivity after remating. The P2’ values (proportion of second male progeny produced after remating) were calculated to test sperm

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The *Drosophila bipectinata* species complex, which comprises of four closely related species, namely *D. bipectinata* (Duda 1923), *D. parabipectinata* (Bock 1971), *D. malerkotliana* (Parshad & Paika 1964) and *D. pseudoanansae* (Bock 1971), may prove to be an interesting model for studying and comparing remating as well as sperm displacement in this species complex\(^{17}\). They are known to occur in the Oriental–Australian biogeographic zones, where all the four species are sympatric over parts of their range\(^{18,19}\). Evolutionary studies based on sexual isolation, degree of crossability, isozyme variations, polytene chromosome morphology and degree of divergence in nuclear and mitochondrial DNA have been done to a great extent in the members of this complex\(^{18,20}\). However, behavioural studies have not been done in detail. The few studies done looked at courtship patterns and mating behaviour in the four species\(^{21-26}\). In our earlier study, we provided evidence that among all the four species, *D. bipectinata* is the most widespread and genetically variable species with respect to remating frequency, latency and duration of copulation in first and second matings\(^{26}\). The frequency of remating is higher and it takes comparatively less time to undergo remating. This helps us to estimate the intensity of sperm competition which may lead to sperm displacement in this species. Similarly, from the fitness point of view, sperm competition may have significant effects on male fitness. In this respect productivity is recognized as a major component of overall fitness and in many cases it is the only one that may provide a direct benefit to females as well as males\(^{9}\). This was however proved by the studies done on *D. ananassae*, where productivity of twice-mated females increases after remating\(^{27}\). Similar type of results was also found in *D. melanogaster*, *D. pseudoobscura* and *D. buzzati*, where females had a productivity advantage after remating\(^{28-30}\). Therefore, in view of the above fact we made an attempt to study the pattern of sperm displacement with respect to productivity in *D. bipectinata*. For studying sperm displacement, paternity pattern (P2') of remated females was analysed using two visible mutant markers (se and ct) and one wild-type strain of this species and the resulting progeny sired by the two males were distinguished based on morphology of the markers.

During the present study, the following stocks of *D. bipectinata* were used: wild-type stock PN (established from a large number of flies collected from Pune in 1999), *se* (sepia eye colour autosomal recessive mutation-II chromosome) and *ct* (cut wing sex-linked recessive mutation-X chromosome). The mating combinations used for control (single matings) as well as for rematings are given in Table 1.

The method of Singh and Singh\(^{27}\) was followed to study sperm displacement in *D. bipectinata*. In control groups (single matings) virgin females and males from the respective stocks (mutant markers and wild type) of *D. bipectinata* were collected and aged for seven days in food vials. A single 7-day-old virgin female was placed in a fresh food vial with a single 7-day-old unmated male and was observed for 60 min. When mating occurred the pair was allowed to complete copulation and the male was discarded within 30 min of completion of copulation. In each cross, 50 mated females were taken for productivity analysis. For testing the productivity, each mated female was kept in an individual food vial for a period of three days and was transferred to a fresh food vial every third day. Three successive changes were made and when the offspring emerged, the total number of flies (male and female) from each vial was counted (crosses 1 and 3). In crosses 5 and 7 only female progeny were counted. In this way productivity of once-mated females was calculated.

In the remating group, 7-day-old females were first mated individually as in control groups (single matings). After the first mating, the females were placed individually in fresh food vials where they were allowed to oviposit for two days. On the third day females were transferred to fresh food vials, along with two virgin males. These flies were kept together for 24 h and then the females were transferred to fresh food vials and the males were discarded (day four). Females were transferred to fresh food vials again on day seven. On day 10, females were again transferred to fresh food vials and on day 12, all females were discarded. In crosses involving

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**Table 1.** Female productivity (mean number of progeny ± SE) in control and remating groups

*Student’s t-test (unpaired) compares total productivity of once- and twice-mated females*

<table>
<thead>
<tr>
<th>Cross</th>
<th>Mean number of progeny (mean ± SE)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>se × PN-control</td>
<td>179.94 ± 13.04</td>
<td>3.23</td>
<td>0.001</td>
</tr>
<tr>
<td>se × PN × se-remating</td>
<td>236.78 ± 12.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>se × se-control</td>
<td>147.45 ± 7.20</td>
<td>10.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>se × se × PN-remating</td>
<td>337.00 ± 16.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ct × PN-control*</td>
<td>83.45 ± 4.20</td>
<td>9.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ct × PN × ct-remating*</td>
<td>146.10 ± 5.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ct × ct-control*</td>
<td>67.63 ± 3.57</td>
<td>11.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ct × ct × PN-remating*</td>
<td>142.32 ± 5.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*For crosses involving X-linked markers only female progeny were taken for analysis.*
Table 2. Mean number of progeny (± SE) of first male (BF1) before second mating and of first male (AFT1) and second male (AFT2) after second mating in different crosses

<table>
<thead>
<tr>
<th>Cross</th>
<th>N</th>
<th>BF1</th>
<th>AFT1</th>
<th>AFT2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>se × PN × se</td>
<td>100</td>
<td>64.39 ± 3.50</td>
<td>70.11 ± 4.84</td>
<td>102.28 ± 7.36</td>
<td>236.78 ± 12.0</td>
</tr>
<tr>
<td>se × se × PN</td>
<td>100</td>
<td>66.80 ± 2.40</td>
<td>89.60 ± 6.26</td>
<td>180.60 ± 12.9</td>
<td>337.0 ± 16.70</td>
</tr>
<tr>
<td>ct × PN × ct</td>
<td>100</td>
<td>31.54 ± 1.30</td>
<td>42.93 ± 2.00</td>
<td>71.63 ± 3.12</td>
<td>146.10 ± 5.30</td>
</tr>
<tr>
<td>ct × ct × PN</td>
<td>100</td>
<td>26.24 ± 1.17</td>
<td>38.50 ± 2.20</td>
<td>77.58 ± 4.0</td>
<td>142.32 ± 5.49</td>
</tr>
</tbody>
</table>

Table 3. P2′ values (proportion of second male progeny produced after remating = AFT2/AFT1 + AFT2) in different crosses

<table>
<thead>
<tr>
<th>Cross</th>
<th>P2′</th>
</tr>
</thead>
<tbody>
<tr>
<td>se × PN × se</td>
<td>0.60</td>
</tr>
<tr>
<td>se × se × PN</td>
<td>0.67</td>
</tr>
<tr>
<td>ct × PN × ct</td>
<td>0.62</td>
</tr>
<tr>
<td>ct × ct × PN</td>
<td>0.67</td>
</tr>
</tbody>
</table>

For each cross of control (single mating) and remating groups, two replicates were carried out. The stocks were maintained on simple yeast-agar culture medium at approximately 24°C with 12 h cycle of light and darkness.

For every cross (control and remating groups), mean number of progeny was calculated and represented as mean ± SE. Student’s t-test (unpaired) was performed to compare the productivity of once and remated females. Similarly, proportion of second male progeny produced after remating (P2′) in different crosses was calculated using the formula of Singh and Singh.

\[ P2' = \frac{\text{AFT2}}{\text{AFT1} + \text{AFT2}} \]

where AFT1 is the mean number of progeny of the first male after second mating and AFT2 is the mean number of progeny of the second male after second mating.

Mean number of progeny produced by once-mated and twice-mated females was calculated. Results of the t-test (unpaired) showed significantly greater productivity for the remated females than that of once-mated females (Table 1). Similarly, mean number of progeny of the first male (BF1) before second mating and of the first male (AFT1) and second male (AFT2) after second mating in different crosses was calculated (Table 2). Females produced more progeny from second male after remating than first male before and after second mating. P2′ values for all crosses vary from 0.60 to 0.67 (Table 3), which extends evidence for the existence of the phenomenon of sperm displacement in *D. bicipinata*.

Remating/multiple mating is increasingly recognized as a pervasive feature of many species. The hypotheses proposed to explain the evolution of remating/multiple mating generally fall into two categories: (1) to gain material benefits through sperm replenishments and (2) to obtain genetic benefits. It is generally accepted that reproductive success of a male primarily depends on the number of females it can inseminate, however, whether females benefit from multiple copulations is still controversial.

Keeping the above points in view, the present work aimed to study sperm displacement with respect to productivity, which is regarded as one of the fitness parameters in *D. bicipinata*. The results clearly depict that remated females show greater productivity than once-mated females, and the P2′ values vary from 0.60 to 0.67 in different crosses. Thus, sperm displacement is not complete in *D. bicipinata*. Our earlier work demonstrated that *D. bicipinata* females take less time to undergo second mating (2.37 days). Therefore, females get less time to use all the sperm for fertilizing the eggs. As a result, both types of sperm (of first and second males) get the opportunity to fertilize the eggs. On the other hand, in *D. ananassae*, remating latency is more, i.e. 7.17 days; therefore, females get a chance to fertilize most of their eggs before remating occurs. Thus, when we compare the proportion of progeny from first male after remating in *D. ananassae* (most widespread species of the ananassae complex) and *D. bicipinata* (most widespread spread species of the bicipinata complex), it is more in *D. bicipinata*.

According to the incomplete storage hypothesis, (i) release of first male sperm from storage occurs after remating due to the presence of second male sperm in the...
utero; a physiological effect of second male seminal fluid or the act of copulation itself, and (ii) incomplete sperm storage after each mating. Since D. bipunctata females sire progeny from both first and second males, it clearly demonstrates that the sperm of first male was not released from the storage organ and as a result due to incomplete storage of sperm of first male, females undergo second mating in order to replenish the storage organ by the sperm of second male. Clearly, separated sperm storage allows females to effectively influence paternity by limiting the number of copulations achieved by a given male. For example, if a female permits first male only one copulation, she creates the potential for a rival male to inseminate the empty organ and sire at least 50% of her offspring. This might be the case with D. bipunctata females. As for the paternity analysis, we used morphological markers and the marker strains with visible mutations generally have lower fitness than the wild type. They may transfer fewer sperms at a slow rate and thereby fewer sperms are stored, and hence in order to replenish the storage organ they undergo second mating.

The phenomenon of sperm competition which leads to sperm displacement has been studied in both natural and laboratory populations of different Drosophila species with varying degrees of sperm displacement. Rapid diversification of sperm precedence traits and processes among three sibling species of Drosophila, i.e. D. melanogaster, D. mauritiana and D. simulans was studied. P2' value was found to be almost similar in all the three cases ranging from 0.79 to 0.88 (ref. 33). D. bifurca females also show high frequency of removal of the sperm of the first males from the female reproductive tract prior to any interaction with the second male ranging from 0.95 to 0.98 (ref. 34). However, it has been reported that last mated male sperm precedence in doubly mated females of D. n. nasuta and D. n. albomicans is not ubiquitous as in both species the first male showed precedence in fertilizing most of the eggs in a doubly mated female.

Apart from increase in productivity after remating which provides direct fitness to the females, remating also lends to generation of genetic variability. While when there is just one mating in a lifetime, competition occurs among sperms of one individual, when mating happens more than once, there is more competition. This increases the contribution of female to the total genetic variation in a population. Thus, in D. bipunctata remating is favoured and sperms of both males are utilized (P2' values range from 0.60 to 0.67), which represents increased productivity of females and/or increased genetic variability (and hence, pre-adult survivorship) of the progeny. This study will be extended to other members of this complex so that their phylogenetic relationship may be discussed in the light of the pattern of remating and sperm displacement.

Carbon footprint of marine fishery: life cycle analysis from Visakhapatnam

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The contribution of marine fisheries in Visakhapatnam at all stages of its life cycle to climate change during 2010–2012 was studied by determining its carbon footprint. Pre-harvest phase consisted of vessel construction and maintenance and provision of fishing gear; harvest phase included harvest from mechanized and motorized craft and post-harvest phase involved fish transportation and fish processing. The functional unit selected was 1 kg of marine fish to the consumer. Fuel and electricity consumption was 0.48 l/kg and 0.255 kWh/kg of fish. The C and CO2 emitted were 0.382 kg C/kg and 1.404 kg CO2/kg of fish. The highest consumption of energy and the highest emissions of CO2 were observed from the harvest phase. The fuel and electricity consumption and C and CO2 emissions were high for mechanized landings and low for motorized landings. Reduction in energy consumption and subsequent emissions is possible in mechanized craft by increasing the fuel efficiency of marine diesel engines, controlling craft speed, using large propeller with lower rpm, and reducing the draft drag.

Keywords: Carbon footprint, CO2 emission, energy consumption, lifecycle analysis, marine fisheries.

As fishing relies entirely on the extraction of organisms from essentially wild ecosystems, most concerns regarding the environmental impacts of fishing have traditionally focused on its direct impacts on targeted stocks13,14, by-catch and discards15, destruction to benthic communities and substrates16,17 and on the general alteration of ecosystem structure and function18. While this focus on biological concerns is understandable given the degraded state of many fish populations and aquatic ecosystems, it is also of paramount importance to study the diverse range of environmental impacts which flow from the interlinked series of industrial activities that characterize most modern fishing systems. These include the material and energy dissipated in the construction and maintenance of fishing vessels9, provision of fishing gear10, combustion of fuel while fishing11–13 and transporting catch to markets or for further processing14, and the discharge of waste and loss of fishing gear at sea15. The best way to evaluate the range of environmental impacts

Acknowledgements. The financial assistance in the form of Meritorious Fellowship to A.S. and UGC-BSR Faculty Fellowship Award to B.N.S. from the University Grants Commission, New Delhi is acknowledged. We also thank the anonymous reviewer for helpful comments on the original manuscript.

Received 9 September 2013; revised accepted 16 June 2014

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