Odonatologica 16(3): 291-296 *September 1, 1987*

HAND-PAIRING: A NEW TECHNIQUE FOR OBTAINING COPULATIONS WITHIN AND BETWEEN *CALOPTERYX* SPECIES (ZYGOPTERA: CALOPTERYGIDAE)

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Received July 21, 1986 / Revised and Accepted February 16, 1987

A method is described for inducing the tandem position by hand-pairing in *Calopteryx* species. In *C. maculata*, hand-pairing resulted in tandem formation and normal copulation in 81 out of 91 pairs. Thus the elaborate pair formation and courtship normally seen in this species are not required for copulation to occur. Conspecific pairs were also obtained for *C. aequabilis*, *C. splendens*, and *C. virgo*, but not for several other species of Zygoptera. 30 of 36 heterospecific pairs between *C. maculata* and *C. dimidiata* resulted in tandem formation and 17 of these ended in copulation, despite a size difference between the two species. This result suggests a lack of mechanical isolation or species discrimination at the tandem and copulation steps.

INTRODUCTION

In the Zygoptera, mating is a two-stage process. Tandem formation is achieved when the male grasps the female's prothorax with his anal appendages. Copulation does not occur until the female bends her abdomen and brings her genitalia into contact with the male accessory genitalia. Mating decisions and species discrimination could occur at either or both of these steps (TENNESSEN, 1982).

During studies of the reproductive behavior of *Calopteryx maculata*, we discovered that if a hand-held male's anal appendages were brought into contact with the prothorax of a hand-held female, the tandem position could be attained. We investigated the probability of copulation resulting from this technique in conspecific pairs of five *Calopteryx* species. We also attempted to form tandem pairs between sympatric *C. maculata* and *C. dimidata*, a smaller species. In the majority of cases hand-pairing resulted in tandem formation and copulation

(Tab. I). Hand-pairing is widely used for obtaining controlled matings in Lepidoptera (e.g. PLATT, 1969). A similar technique has been attempted with Aeshnids, but the pairs usually separated (OBANA, 1979). In this paper we describe the hand-pairing technique, present results of conspecific and heterospecific pairing attempts, and discuss a number of questions raised by our results.

METHODS

Figure 1 shows the stages of hand-pairing with a marked pair of *Calopteryx maculata*. A male and a female are caught in a net, and the female is held by her folded wings and allowed to perch on a finger or thumb. Giving the female a perch seems to minimize her struggling. The male is held in a similar position in the other hand. With the male's abdomen held perpendicular to the female's prothorax, the tips of the superior anal appendages are pressed against the female's prothorax. At this point the claspers are usually spread, and the male attempts to grasp the female. In order to get the male's abdomen into position, it is sometimes necessary to maneuver it with a finger. Once tandem linkage appears to be formed the male's and then the female's wings are slowly released. At this point the damselflies usually copulate, either on a finger or after flying to nearby vegetation.

We attempted to hand-pair 91 pairs of *C. maculata* at four field locations (Mill River at Sleeping Giant State Park, north of Hamden (New Haven Co.), Connecticut; Palmer River, south-west of Rehoboth (Bristol Co.), Massachusetts; Flat River, east of Escoheag (Kent Co.) Rhode Island and Squannacook River, south of Townsend (Middlesex Co.), Massachusetts) from 12 July to 14 August

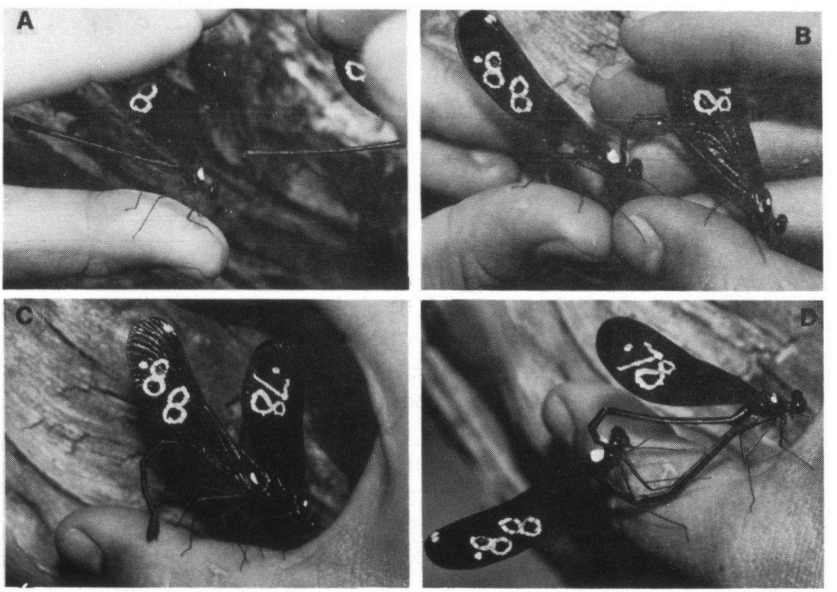

Fig. 1. Stages in the hand-pairing of *C. maculata:* (A) the male and female are held in position for hand-pairing; — (B) the male's anal appendages are pressed against the female's prothorax; — (C) sperm translocation; — (D) copulation.

1985 and 14 June to 12 August 1986. For the hybridization study, we attempted an additional 7 conspecific and 36 heterospecific pairings of *C. maculata* and *C. dimidiata* on the Palmer River.

RESULTS

HAND-PAIRING OF *CALOPTERYX MACULATA*

81 of the 91 pairs of *C. maculata* which we attempted to hand-pair formed the tandem position (Tab. I). Of these, nine broke tandem when released. In most cases this appeared to be due to our releasing the wings too quickly. In four cases the female appeared to resist sperm transfer by grasping a finger or vegetation.

Table I

Numbers and percentages of hand-pairs resulting in tandems, attempted copulations by females, and completed copulations. There was no statistical difference for either species on the Palmer river in the likelihood of a male's pairing with a con- or heterospecific female (G = 0.1963 for *C. maculata* males, G = 1.839 for *C. dimidiata* males) — See text for other comparisons

Male	Female	n	Tandem	Copulation attempt	Copulation
C. maculata	*C. maculata*	43	36	29	29
C. maculata	*C. maculata**	48	45	39	39
C. maculata	*C. dimidiata**	21	19	16	9
C. dimidiata	*C. dimidiata**	7	2	2	2
C. dimidiata	*C. maculata**	15	11	8	8

* Palmer river

The remaining 68 copulated, with sperm translocation preceding copulation in all but one case. Copulation was accompanied by the usual series of abdominal movements described by MILLER & MILLER (1981) and WAAGE (1979a). The average copulation duration for 21 of these pairs was 76.8 ± 11.8s (95% CL) which is comparable to the 79s average copulation duration for natural pairs (WAAGE, 1979a). Thus copulation appears to proceed normally in hand-paired *C. maculata.*

HYBRIDIZATION STUDY

The results of the conspecific and heterospecific pairings on the Palmer River are shown in Table I. Thirty of the 36 heterospecific pairings resulted in tandem formation. For both species, there was no significant difference in the likelihood of a male's forming the tandem position with a con- or heterospecific female. The tandem formation rate with both conspecific and heterospecific females was

lower overall for *C. dimidiata* males than for *C. maculata* males ($G = 11.848$, $p < 0.001$). *C. maculata* males were more likely to grasp *C. dimidiata* females than were *C. dimidiata* males ($G = 8.910$, $p < 0.005$). This appeared to be due to failure of the males to open their claspers, and not to any behavior by the females.

Once tandem was achieved, the female usually attempted to copulate by curling her abdomen and making contact with the male genitalia. Copulation, however, did not result from such attempts in seven of the male *C. maculata*-female *C. dimidiata* pairs. In these cases, it seemed that the larger size of the *C. maculata* genitalia prevented the female from forming the wheel position. In those pairs in which copulation did occur, it appeared to be initiated with difficulty and the genitalia often separated several times during copulation. In contrast, female *C. maculata* which attempted to copulate with *C. dimidiata* were always (8 of 8) able to do so. Thus it appears that *C. maculata* females are more capable of copulating with *C. dimidiata* males than are *C. dimidiata* females with *C. maculata* males, though with this sample size the difference is not significant ($p = 0.0538$, two-tailed Fisher's exact test).

HAND-PAIRING IN OTHER SPECIES

We attempted the hand-pairing method on three other species of *Calopteryx*. These included one pair of *C. aequabilis* in Connecticut, three pairs of *C. splendens* in England, and one pair of *C. virgo* in France. Each case resulted in tandem formation and copulation. In addition, two of two *C. aequabilis* females mated after hand-pairing with *C. maculata* males, and seven of eight *C. maculata* females mated after hand-pairing with *C. aequabilis* males.

We also attempted the technique with several other species of Zygoptera: several pairs each of *Argia fumipennis violacea, Ischnura* sp. and *Lestes* sp. In no case were we able to obtain tandem formation, though the *Lestes* males would open their claspers.

DISCUSSION

WAAGE (1984) found that only 30% of *C. maculata* courtships end in copulation. In contrast, 81 of 91 (89%) of our attempted hand-pairings of *C. maculata* resulted in tandem formation and 68 (84%) of these led to copulation. Thus hand-pairing appears to bypass aspects of natural pair formation and courtship that lead to rejection of courting males. The fact that hand-paired females are more likely to mate than natural ones indicates that courtship displays in this species may not be necessary for inducing copulatory behavior.

Because most tandem pairs copulated in our hand-pairing attempts, it would seem that it would be adaptive for males to grasp females without courting them. However, two things should be taken into account. First, tandem without courtship does occur in this species, in particular when females are ovipositing

(WAAGE, 1974). These tandems usually do not lead to copulation as a female so grasped often will not let go of the oviposition substrate and the male eventually releases his hold. Second, we have insufficient data on oviposition and postcopulatory behavior after hand-pairing to judge whether or not females are as likely to oviposit when they have not been courted as when they have.

Our attempts at hand-pairing *C. dimidiata* were less successful and the number that resulted in tandem (2 of 7) was similar to natural results of courtship (22 of 93, WAAGE, 1984). It is interesting to note that the *C. dimidiata* hand pairs which failed to result in tandem did so as a result of male behavior (failure to open the claspers), and not from any female behavior which we could see. Indeed female readiness to mate in this species can be inferred from the results of pairing them with *C. maculata* males, in which 16 of 21 pairings resulted in attempted copulation by the female.

Heterospecific tandem formation and matings are known to occur between many odonate species (summarized in BICK & BICK, 1981). In *Calopteryx*, hybridization, including oviposition, has been observed between *C. maculata* females and *C. aequabilis* males (WAAGE, 1975) and between a *C. maculata* male and *C. dimidiata* female (pers. obs.) and a *C. maculata* female and a *C. dimidiata* male (D. Schoeling, pers. comm.). Copulation, with possible oviposition, has also been reported between two female *C. maculata*-male *Hetaerina americana* pairs (WEICHSEL, 1985), and MOORE (1953) reports tandem formation between male *C. splendens* and female *Platycnemis pennipes* (Platycnemididae).

Our hybridizations and these natural ones contrast strongly with the genera *Lestes* and *Ischnura* (LOIBL, 1958; KRIEGER & KRIEGER-LOIBL, 1958) in which females taken in tandem by heterospecific males refused to copulate, and for *Enallagma* species (PAULSON, 1974) in which males offered live pinned females of several species exhibited a range of isolation at the tandem step. TENNESSEN (1975) found that two species of male *Enallagma* attempting to grasp heterospecific females are rejected by those females, apparently on the basis of the shape of their anal appendages. TENNESSEN (1975) and ROBERTSON & PATERSON (1982) found that male *Enallagma* with altered anal appendages were rejected by conspecific females. In each of these genera there is considerable variation between species in the shapes and sizes of anal appendages and no courtship. In *Calopteryx* there are pronounced pairforming and courtship displays of the wings by males. Different species vary in wing coloration but their anal appendages, though somewhat varied in size, are similar in shape (WAAGE, 1975). Thus our preliminary results agree with the prediction that genera in which males are differently colored will be visually isolated while those in which male anal appendages differ in shape will be isolated mechanically or by lack of tactile recognition by heterospecific females (PAULSON, 1975; WILLIAMSON, 1906).

ACKNOWLEDGEMENTS

We thank GEORGE GILCHRIST, BASTIAAN KIAUTA, JOEL KINGSOLVER, DOUG MORSE, and DIANE WIERNASZ for their comments, and PHIL ROBAKIEWICZ for his comments and expert field assistance. We also thank KARI NELSON and JEREMY STRAUGHN for their assistance, and we thank KIYOSHI INOUE for kindly describing SHIGERU OBANA's work. This study was supported by NSF gant BSR 8400171 to JKW.

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