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IN SITU FIXATION OF INTACT TANDEM LINKAGES OF ZYGOPTERA IN THE FIELD

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By means of the fixation of different copulation stages of 17 European species (16 Zygoptera, 1 Anisoptera) the possible use and limitation of a new fixation method, using chloroethane, is described. Only in 2 spp. did the fixation attempts fail. In 15 cases the contact points of the tandem linkage could be analysed. The best phase for fixation is the wheel position; only in *Lestes* and some Coenagrionidae is the postcopulatory tandem suitable as well. If both the inferior and superior male appendages act like forceps, as in *Calopteryx*, only a few attempts are necessary to yield fixations of high quality. In other species up to ten pairs are required to achieve satisfactory results to evaluate the contact points of the tandem linkage.

INTRODUCTION

The tandem linkage and the secondary copulatory apparatus are basic parts of the mating system in Odonata. The secondary copulatory apparatus has been the object of extensive investigations in both morphology (e.g. SCHMIDT, 1916; PRASAD, 1988) and function (e.g. WAAGE, 1979; PFAU, 1971, 1991), whereas the tandem linkage of Odonata has been investigated less intensively. Only the morphology of the structures (the male anal appendages and, in Zygoptera, the female thorax) which establish this connection between male and female, have been analysed (e.g. DUMONT, 1974, 1977; JURZITZA, 1975; JUSTUS et al., 1990; HÄMÄLÄINEN, 1991; GARRISON, 1994, 1996). Almost the whole of our knowledge of the function of the male anal appendages and the female thorax during mating is based on the investigation of intact tandem linkages in *Ischnura elegans* (SCHMIDT, 1967), *Ischnura fluviatilis* and *Oxyagrion rufulum* (JURZITZA, 1974), *Coenagrion scitulum* (BOULARD, 1981) and *Enallagma glaucum* (ROBERTSON & PATERSON, 1982). DUMONT & BORISOV (1993) acquired the probable contact points between the male anal appendages and the female tho-

rax in *Sympecma* sp. without having available an intact grasp. Previous attempts to study the contact points during tandem linkage in Odonata have not led to detailed information (WILLIAMSON, 1906; TILLYARD, 1917). Most of the examined intact links are based on stability by chance. Only ROBERTSON & PATERSON (1982) have attempted to fix the tandem linkage after capturing the pairs; this they achieved by dipping them into liquid nitrogen.

The lack of an easy method for fixation of tandem linkages is the main reason for the slow growth of knowledge in this topic. Therefore, a suitable method, developed to be usable in fieldwork, is presented here.

MATERIAL AND METHODS

Mating pairs of dragonflies were caught carefully with a little hand-held net (20 x 12 cm, 15 cm deep; strengthened by wire) and frozen by spraying with chloroethane (CH_3-CH_2-Cl, medical freezing spray: Chloraethyl „Dr. Henning"). Alternatively, pairs sitting in vegetation were frozen directly, while a net, which was held diagonally below, caught the pair if necessary. The spraying distance varied between 2 and 5 cm. At the beginning of freezing the centre of the spraying mist was pointed at the female thorax. Then the mist was extended above the whole pair, so that both are frozen in a split second. After three or four seconds the spraying was stopped. The pair of dragonflies was gripped immediately with a pair of tweezers after hoarfrost formation, and placed in a sampling tube while still in the frozen state. The animals were usually gripped by the wings of the female but occasionally by the female abdomen or synthorax. The samples were dried in the sun for a minimum of two hours either in the field or in the car. Transport took place after the samples were nearly dry. During transport the sampling tubes were packed in little plastic boxes and placed on a cushion to protect them from movement.

In 16 Zygoptera and one Anisoptera (Tab. I) the attempt was made to preserve intact tandem linkages. In the case of the two *Calopteryx* species, precopulatory pairs were obtained by hand-pairing (OPPENHEIMER & WAAGE, 1987). In all cases, except the two Calopterygidae and the Libellulidae *Sympetrum danae*, the main emphasis was placed on fixation of postcopulatory pairs. Within the Platycnemididae and the Coenagrionidae copulatory pairs were used more often as basis for the fixation. Precopulatory tandems were only caught in a few species (Tab. I).

For the investigation of the tandem linkage points the specimens were embedded into conductive carbon, the coarsest dirt removed with a minute needle, coated with gold, and examined with a scanning electron microscope (SEM).

RESULTS

The suitability of the three mating phases as a starting point for fixation is different between the species. No losses occurred during the capture of wheel positions in any of the *Lestes* species tested, in *Platycnemis pennipes*, *P. latipes* and *Cercion lindenii*, nor in postcopulatory tandems in *Lestes viridis* and *L. dryas* (Tab. I). In *Ceriagrion tenellum* the method failed; all frozen pairs lost contact after thawing. The capture of postcopulatory tandems of *Lestes virens* and *L. sponsa* was associated with some losses of intact linkages. The capture of postcopulatory tandems of the *Platycnemis* species resulted in many futile attempts and all examined

Coenagrionidae showed a high loss in this phase of fixation (Tab. I). Losses during drying always concerned postcopulatory tandems. Under the described transport conditions most of the samples survived long distances (up to 1500 km) by car (Tab. I). Only in *Erythromma viridulum* and *Sympetrum danae* was there a complete loss during transport. Besides, in *S. danae* the male anal appendages displaced after losing contact because of insufficient drying.

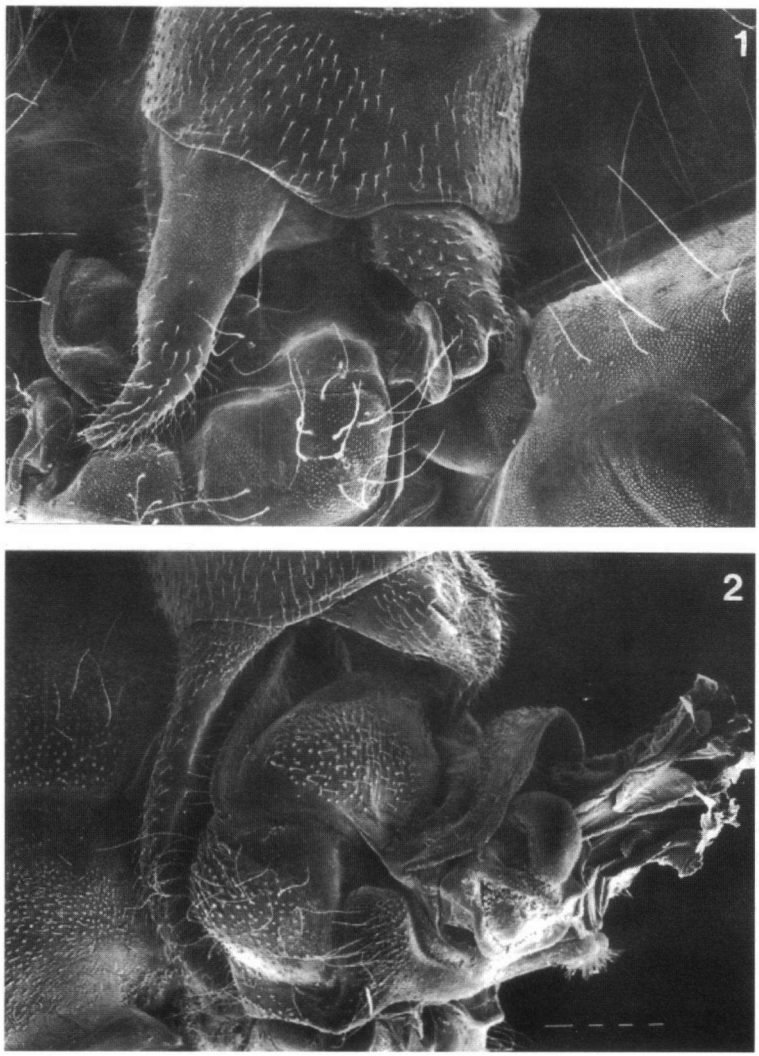
The quality of the preparations depends on both the function of the male abdominal appendages of the species and the mating phase which was used as the starting point for the fixation. All fixation attempts of *Calopteryx* species yielded preparations of high quality similar to those of *Platycnemis* species captured in the wheel position (Fig. 1). Within the genus *Lestes* the results show no uniformity. In *Lestes dryas* the clasp of the appendices is very stable in all fixed postcopulatory tandems, whereas in those species which have short inferior appendages (*L. viridis*, *L. barbarus* and *L. virens*), the tip of the male abdomen is often more or less dorsocaudally displaced if caught in copulation (Fig. 2), or in precopulatory (Fig. 3) or postcopulatory tandem. However, in all these cases the quality of the fixation is sufficient to discern the functional connection of the linkage. A special problem

Table I

The number of pairs caught in the different copulation phases and the loss of intact linkages during the different fixation stages (in number of losses/number of fixations); [ ] = hand-pairing

Family and species	Pre-copulation	Capture Copulation	Post-copulation	Drying	Tranport
CALOPTERYGIDAE					
Calopteryx haemorrhoidales	[0/1]	-	-	0/1	0/1
Calopteryx splendens	[0/2]	-	-	0/2	0/2
LESTIDAE					
Lestes viridis	-	0/1	0/2	0/3	1/3
Lestes barbarus	-	0/1	0/1	0/2	0/2
Lestes virens	1/2	0/1	1/5	0/6	0/6
Lestes sponsa	0/1	0/2	2/6	0/7	0/7
Lestes dryas	-	-	0/5	0/5	0/5
PLATYCNEMIDIDAE					
Platycnemis pennipes	-	0/3	2/5	1/6	0/5
Platycnemis latipes	0/1	0/5	4/6	2/8	1/6
Platycnemis acutipennis	-	-	6/8	0/2	0/2
COENAGRIONIDAE					
Erythromma viridulum	-	1/3	3/3	0/2	2/2
Coenagrion mercuriale	-	-	3/8	1/5	1/4
Cercion lindenii	-	0/2	3/6	0/5	0/5
Enallagma cyathigerum	1/1	-	1/3	0/2	0/2
Ischnura elegans	-	3/8	-	0/5	0/5
Ceriagrion tenellum	-	5/5	-	-	-
LIBELLULIDAE					
Sympetrum danae	0/1	-	-	0/1	1/1

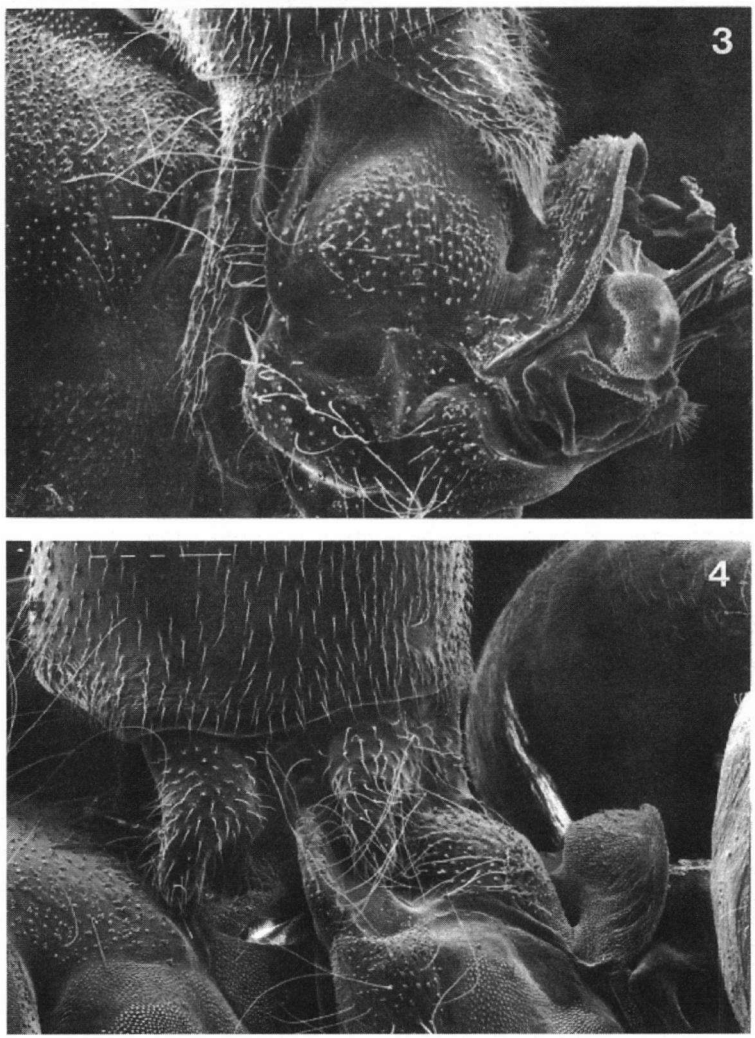
occurred in *Lestes sponsa*, due to the involvement of the females head in the tandem linkage. In this species seven pairs were fixed, but in only three of them the head did not become displaced after thawing. Indeed in no case in all the fixed pairs of Zygoptera did the head arrester system fix the female head as depicted by GORB (1993). Within the Coenagrionidae the inferior appendages especially lost



Figs 1-2. Tandem linkage in *Platycnemis latipes* and *Lestes barbarus*: (1) high quality fixation of *P. latipes*, fixed in the wheel position; left lateral view; – (2) acceptable quality fixation of *L. barbarus* in the wheel position. The tip of the male abdomen shows a dorso-caudal displacement; right lateral view.

contact during fixation. In the species of this family the quality of the fixation results varies more than in other families. In several species, e.g. *Coenagrion mercuriale*, *Cercion lindenii* and *Enallagma cyathigerum*, the fixation of postcopulatory tandems was, in some cases, of excellent quality (Fig. 4).

Whereas the quality of the fixation of the complex of the male appendices and the female thorax varies more or less between and within the species, even when



Figs 3-4. Tandem linkage in *Lestes virens* and *Coenagrion mercuriale*: (3) acceptable quality fixation of *L. v. virens*, fixed as a precopulatory tandem; right lateral view; – (4) high quality fixation of *C. mercuriale*, fixed as a postcopulatory tandem; right lateral view.

fixed in the wheel position, the contact between the secondary copulatory apparatus and the ovipositor during copulation has always been preserved with high quality (see JÖDICKE, 1996 for figure). In one fixation attempt a postcopulatory tandem was coincidentally fixed during oviposition (Fig. 5).

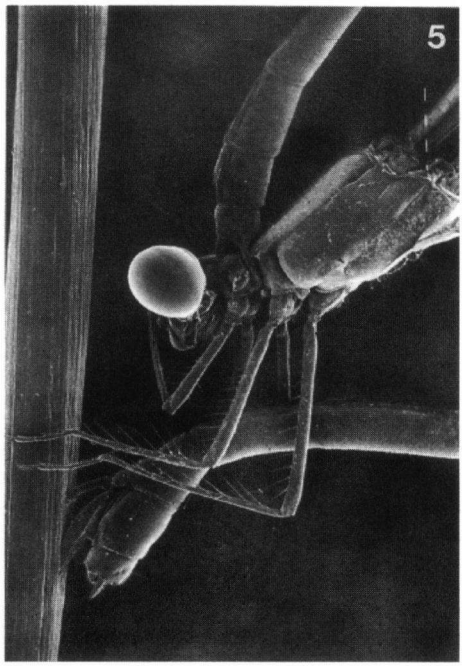


Fig. 5. Tandem of *Lestes virens vestalis* during oviposition in *Juncus* sp.

DISCUSSION

The method was convincing in both its feasibility and in quality of the results. The chemicals which were selected for freezing are very effective in fixing the tandem linkage in Zygoptera. The fixation of the sampling tubes and the use of a cushion as an additional shock absorber during transport can be regarded as sufficient safety devices, due to the few losses of intact linkages during transport. Alternatively the dried specimens could be stored in little envelopes which were packed into a small box to prevent movement (Inoue, pers. comm.). Couplings packed in this manner survived transport by airmail without any damage. This, and the minimal equipment which is needed for capture, recommend this method for fieldwork rather than freezing in liquid nitrogen (ROBERTSON & PATERSON, 1982), which is not available everywhere.

The most futile attempts occurred, especially in *Platycnemis*, by using postcopulatory tandems for fixation. In this phase the active role of the male is finished and it has only to follow the movements of the female during oviposition. This suggests that, in this mating phase, the grasp of the male is not so firm as in the wheel position, or, under consideration of the female recognition system (LOIBL, 1958; KRIEGER & KRIEGER-LOIBL, 1958) which needs a stable grasp to receive stimulation by the male, in the precopulatory tandem. Another argument in favour of using copulations for the fixation is based on statics. In this phase the pair is connected at two points and they cannot twist against each other during transfer into the sampling tube. The restriction on copulations and, maybe, precopulatory tandems as starting points for the fixation should reduce the number of futile attempts utilising this method. If both the inferior and superior appendices act like forceps, as in *Calopteryx*, only a few attempts are necessary to yield fixations of high qual-

ity. However, it is often necessary to fix five pairs within a species in order to compensate for the variation in quality and possible losses of usable specimens during processing for the SEM. The number of samples required increases with the morphological complexity of the tandem linkage and the reduction of the male abdominal appendages. The elucidation of the grasp of the superior appendages under the pronotal hind margin in some Coenagrionidae (e.g. *Coenagrion*) demands just as many additional fixations as an attempt to fix species with dainty male abdominal appendages (e.g. *Ischnura elegans*). Up to ten fixed pairs are required to achieve a satisfactory evaluation of the contact points of the tandem linkage in such cases. In species in which the female head is involved in the tandem linkage (e.g. *Lestes sponsa*) it has to be guaranteed that the head cannot twist itself after thawing.

The fixation results in *Ceriagrion tenellum* mark the limitation of this method, which is founded in morphology and function of the male abdominal appendages. It will be extremely difficult to fix the tandem linkage in species which have very short male abdominal appendages, as in *Ceriagrion tenellum*, that lose contact after thawing. Of course, it is in principle possible to preserve the tandem linkage in such species. The main problem is the prevention of thawing during transportation, whereas the following lyophilisation should not be a problem. In such cases in which the clasp of the male abdominal appendages loses contact during transport (e.g. *Erythromma viridulum*), this problem may be solved by gluing the samples on before transport. However, this is only required if the existence of an intact tandem linkage is essentially to investigate the contact points. Since the specimens have to be dried before transport, the male abdominal appendages are fixed in the working position and the contact points with the female thorax can easily be investigated.

The result in *Sympetrum danae* indicates, that it is also possible to use this method to fix at least small anisopteran species. Whether the genital contact of Anisoptera can be fixed by this method has to be tried out in future. The good results in fixing Zygoptera during mating and oviposition suggest that the presented method has a high potential to enable access to those parts of insect life which usually evade observation.

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