

**PRECOPULATORY REPRODUCTIVE ISOLATION AND WING COLOUR DIMORPHISM IN *CALOPTERYX SPLENDENS* FEMALES IN SOUTHERN ITALY (ZYGOPTERA: CALOPTERYGIDAE)**

G. DE MARCHI

Via Hermada 15, I-20162 Milano, Italy

*Received April 11, 1989 / Revised and Accepted March 14, 1990*

Interspecific interactions were studied in 3 localities, where there are *C. splendens* populations with 2 female forms. The homeochromic form, possibly more vulnerable to predation because of its conspicuousness, gains advantage over the heterochromic form because it offers a better reproductive isolation. In fact homeochromic females sexually attract heterospecific males less often than the other females do and so are probably less often involved in heterospecific matings. This is the result of their wing colouration, variable according to the congeneric species sympatric in the single sites, which mimics the wings of *C. haemorrhoidalis* males and which is very different from those of *C. virgo* females.

**INTRODUCTION**

In some odonate species there is a female dimorphism with a homeochromic form which has a bright colouration like males, and a heterochromic form with a more cryptic colouration. Only few species showing this phenomenon were studied from an ecological point of view and different selective pressures involved in the maintenance of the dimorphism were discovered.

JOHNSON (1975) studied the North American *Ischnura damula* and *I. demorsa*, whose homeochromics are very different from each other, while the heterochromics are similar. Unlike heterochromics, homeochromics are never wrongly identified and taken into tandem by males of the other species. On the other hand, he found that homeochromic females were more vulnerable to predation. He also demonstrated that the dimorphism in these species is controlled by a single autosomal locus and homeochromics are the recessive homozygotes (JOHNSON, 1964, 1966).

ROBERTSON (1985) studied *Ischnura ramburi*, another North American

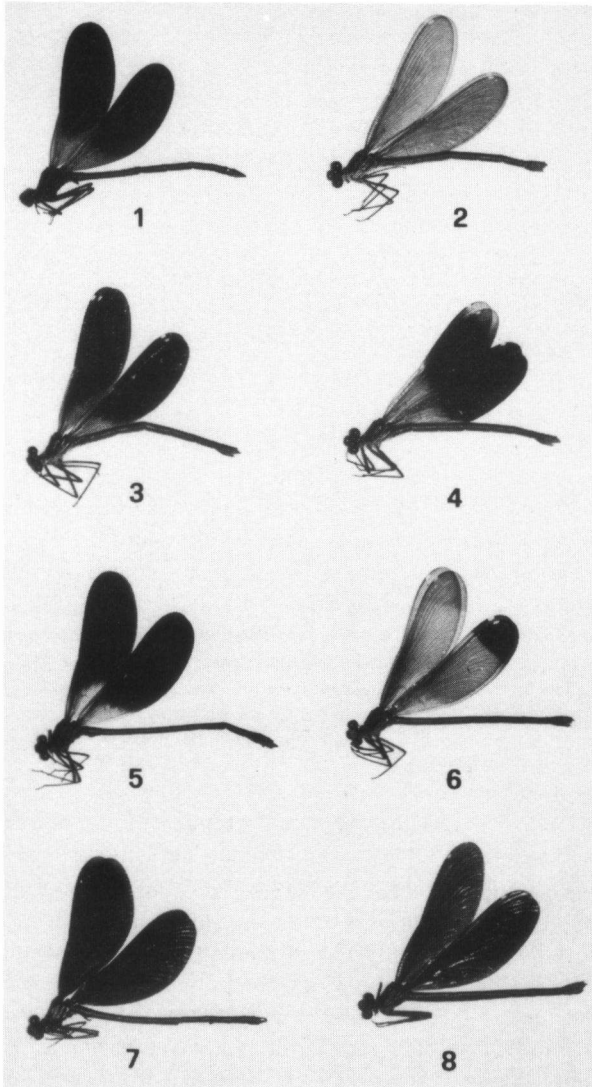


Fig. 1. Colouration types occurring in the populations studied: (1-4) *Calopteryx splendens*: (1) male, — (2) heterochromic female, — (3-4) homeochromic female; — (5-6) *C. haemorrhoidalis*: (5) male, — (6) female; — (7-8) *C. virgo*: (7) male, — (8) female. — [Nos 1-3, 5-6 from loc. 3, — Nos 4, 7-8 loc. 1]

species which was not sympatric in the study area with any other species that could have been the cause of reproductive interference. He discovered that homeochromics, when not sexually receptive, can easily avoid males because of

their appearance and by acting in an aggressive way when approached by males. They mate only half as often as heterochromics. This fact represents an advantage for a female who has already mated and still has a sperm reserve in her spermatheca as she avoids wasting time and increased predation during unnecessary matings, which last many hours in this species, as in other *Ischnura*.

HINNEKINT & DUMONT (1989), reinterpreting published information regarding sexual and aggressive behaviour of the European *Ischnura elegans*, suggested that homeochromic females are favoured in dense populations, escaping the attention of males in such a way that they can maintain themselves near the water and lay eggs more easily. On the other hand they are disadvantaged in sparse populations as they are less attractive to males.

A female dimorphism also occurs in some populations of the *Calopteryx splendens*-group in Europe (DUMONT, 1971) and in the Middle East (BUCHHOLTZ, 1955). Besides hyaline winged females, there are homeochromics which have a male-like coloured spot on the wings, though they still differ from the males by the presence of a white pterostigma and by the green rather than blue body coloration. Moreover, the wing spot in the homeochromics is brown, not blue, and its extension is generally different from that in the males. The exception are some populations in Yugoslavia, where homeochromics occur with blue wings and bodies (FUDAKOWSKI, 1930; DUMONT, 1977). The generic basis of this dimorphism is still unknown.

The reproductive behaviour of *C. splendens* has been studied by various workers (e.g. ZAHNER, 1960; HEYMER, 1972, 1973), but no evidence is available on homeochromics from an ethological point of view.

In order to fill this gap I have carried out observations and experiments in southern Italy, where there are populations of *C. splendens caprai* showing this dimorphism. The results suggest that the homeochromic form gains advantage over the heterochromic form when *C. splendens* is sympatric with congeneric species, because this form allows an increased reproductive isolation.

#### STUDY AREA AND METHODS

Observations on the reproductive behaviour of three *Calopteryx* species were performed during 19 days in June and July 1986 and June and July 1987 in the following sites: Acqua dell'imperatore River (locality No. 1) (40°18'N, 3°11'E) and Calore River (loc. No. 2) (40°15'N, 3°11'E) both near Montesano scalo, Campania, and Esaro River (loc. No. 3) (39°42'N, 3°51'E) near Spezzano Albanese scalo, Calabria.

In order to allow individual recognition of observed specimens in loc. No. 1, 31 males and 30 females of *C. splendens* were marked with green spots on hind wings and abdomen.

In order to study the ability of males to distinguish their own females from those of other species a slightly modified version of MOORE's (1952) fishing line technique was used. A living female is connected to a thin stem (length 1 m) by a nylon fishing line tied to her thorax; a little weight is placed on the thread 20 cm over the damselfly in order to limit its flight upwards. With an appropriate handling of the stem the female is allowed to fly for a few seconds in the territory of a male but she is

prevented from perching. This flight mimics the female natural behaviour when she enters the male territory in search of an oviposition site. Male reaction is classified as sexual if he takes off from his perching site and performs the typical courting flight (RÜPPELL, 1985), otherwise the reaction is considered non-sexual. Sometimes non-sexual reaction was a typical aggressive territorial flight (RÜPPELL, 1985). Data obtained with the fishing line technique agreed with observations of spontaneous interactions which, nevertheless, were hard to quantify.

## RESULTS

Homeochromic females represented 10% of the total population of *C. splendens* females in loc. 3 (n=59), 13% in loc. 1 (n=31) and 15% in loc. 2 (n=61).

Homeochromic females were observed for many hours during feeding and reproductive activities mainly in loc. 1 and loc. 2, but no behaviour different from that of heterochromics could be noticed. In particular, they never reacted in an aggressive way when approached by males.

Figure 1 shows the appearance of males and females of the three congeneric species found in the study sites: *C. splendens caprai*, *C. v. virgo* (sensu MAIBACH, 1986, 1987; previously *C. virgo padana*), and *C. h. haemorrhoidalis*. In loc. 1 (n=79) *C. splendens* (36%), *C. virgo* (60%) and *C. haemorrhoidalis* (4%) were present; in loc. 2 (n=75) *C. splendens* (69%), *C. virgo* (30%) and *C. haemorrhoidalis* (1%); in loc. 3 (n=197) *C. splendens* (80%) and *C. haemorrhoidalis* (20%).

Interspecific interactions were very common because all species undertook sexual behaviours during the central hours of the day. Interspecific territorialism was present, but less developed than the intraspecific one. For example, two conspecific males never share the same territory, while heterospecific males often do. In loc. 1, where in June 1986 the population density was very high (over 90 *Calopteryx* males every 100 meters of river), over 50% of *splendens* males shared with *virgo* males.

In loc. 3, *splendens* and *haemorrhoidalis* males were observed sharing the same territory.

Interactions between *virgo* males and *haemorrhoidalis* males were greatly influenced by the fact that the former courted the latter, clearly mistaking them for conspecific females. A *virgo* male has been observed flying in tandem with a *haemorrhoidalis* male in loc. 1, though this tandem broke after a very short while. [It is easy to get this kind of tandems with the fishing line technique, introducing a *haemorrhoidalis* male into the territory of a *virgo* male]. Interactions among males and females of different species were also common.

Table I shows the results of experiments performed with the fishing line technique. In particular it could be noted that *splendens* males courted homeochromics and heterochromics with the same frequency ( $\chi^2=0.31$ ;  $p>0.5$ ). This agrees with the evidence on marked males that were observed courting and mating with both female morphs. It seems therefore that there are not two male

forms, each one with a preference for one female form.

*C. splendens* males courted females of the other species with the same frequency but less commonly than conspecific females ( $\chi^2=24.7$ ;  $p < 0.001$  when heterospecific females are compared with heterochromics and  $\chi^2=38$ ;  $p < 0.001$  when compared with homeochromics).

*C. virgo* males courted *haemorrhoidalis* females as often as conspecific ( $\chi^2=0.017$ ;  $p > 0.5$ ) and *splendens* females less than conspecific females ( $\chi^2=69$ ;  $p < 0.001$  for homeochromics and  $\chi^2=48$ ;  $p < 0.001$  for heterochromics). Nevertheless they courted heterochromics more than homeochromics and this difference is nearly significant ( $\chi^2=3.5$ ;  $0.1 < p < 0.05$ ).

Table I

Reaction of territorial males of three *Calopteryx* species to females introduced into their territories with the fishing line technique — [n = number of tested males; — He = heterochromic females; — Ho = homeochromic females; — % sex = per cent of sexual reaction; — % non-sex = per cent of non-sexual reaction]

Males	Females	N	% sex.	% non-sex.
<i>splendens</i>	<i>splendens</i> (Ho)	78	85	15
	<i>splendens</i> (He)	46	80	20
	<i>virgo</i>	33	24	76
	<i>haemorrhoidalis</i>	33	24	76
<i>virgo</i>	<i>splendens</i> (Ho)	71	11	89
	<i>splendens</i> (He)	69	23	77
	<i>virgo</i>	32	97	3
	<i>haemorrhoidalis</i>	20	95	5
<i>haemorrhoidalis</i>	<i>splendens</i> (Ho)	27	7	93
	<i>splendens</i> (He)	28	71	29
	<i>haemorrhoidalis</i>	11	100	0

*C. haemorrhoidalis* males courted homeochromics much less frequently than both heterochromics ( $\chi^2=23.6$ ;  $p < 0.001$ ) and conspecific females ( $\chi^2=29.8$ ;  $p < 0.001$ ). *C. haemorrhoidalis* males very often (70%) performed aggressive territorial flights when in presence of homeochromics. The few cases in which they reacted with courting flights happened when homeochromics were introduced to males which were sexually excited because of the recent arrival of conspecific females. Because of the scarcity of *haemorrhoidalis* males living with *virgo* in the study sites, the fishing line technique could not be used to test their reaction to *virgo* females. The few observations of spontaneous interactions showed that there was almost always courting behaviour.

A hybrid mating has been observed in loc. 1: a heterochromic *splendens* female, after an underwater oviposition in a territory of a *virgo* male, surfaced and was soon taken in tandem without being courted; she curved her abdomen under her body, bringing it in contact with male genitalia and mated.

It is easy to get tandems and hybrid matings with a simulation of this situation, by placing a female with wetted wings, so that she can not fly easily, on the oviposition site in a territory belonging to a heterospecific male.

A few, probably interspecific hybrids, now under study, were found in the same locality.

## DISCUSSION

Males of the three *Calopteryx* species found in southern Italy differ from each other in the extent and coloration of wing spots and in the colour of the three posterior urites. These characters are displayed during the courtship, when females enter the male territories. On the basis of these characters, females identify, accept and mate only with conspecific males (HEYMER, 1973; personal observations). Courting is not performed when a male discovers an egg-laying female in his territory, but he often succeeds in landing on her wings and in taking her in tandem which she cannot avoid (WAAGE, 1973; personal observations).

Consequently, it seems that a hybrid tandem can be formed when a male finds a heterospecific female ovipositing in his territory and he fails to identify her. These meetings occur particularly when, because of a high population density, two males of different species hold the same territory, and females they mate with use the same oviposition site. During tandem, at least in Zygoptera, male abdominal appendices contact sensitive areas on the female's prothorax, stimulating her to mating (TENNESSEN, 1982; ROBERTSON & PATERSON, 1982; HILTON, 1983). Once caught in tandem a *Calopteryx* female is stimulated and mates also with heterospecific males (OPPENHEIMER & WAAGE, 1987) because in *Calopteryx*, unlike in many other Zygoptera, the male abdominal appendices of different species are similar.

The experiments performed with the fishing line technique show that in southern Italy there is reproductive interference among the three species and in particular that the interference caused by *haemorrhoidalis* and *virgo* males is stronger against heterochromics than against homeochromics. Reproductive interference favouring homeochromics and (still to be tested) predation favouring heterochromics could be the selective pressures involved in the maintenance of wing dimorphism in *C. splendens* as found by JOHNSON (1975) in the species he studied.

Reproductive interference between congeneric species can be the selective pressure for a character displacement (Grant, 1972). One case of this kind regarding the colour of wings has been demonstrated in two *Calopteryx* species by WAAGE (1975, 1979).

It is possible that character displacement also took place in southern Italy as suggested by the following considerations. In spite of the individual variability in the single populations there is a great difference in wing colouration between

homeochromics found in loc. 3 and those found in localities 1 and 2 (Fig. 1). Homeochromic females of loc. 3, which cohabit with *C. haemorrhoidalis*, almost always have a wing spot mimicking that of *haemorrhoidalis* males, evidenced by extension and brown colouration. The more they resemble *haemorrhoidalis* males, the less they stimulate their sexual interest. This mimicry is shown to be effective by the usually aggressive reaction performed by *haemorrhoidalis* males as if they were in presence of males.

Homeochromics of localities 1 and 2, where *splendens* mainly co-occurs with *virgo*, differ from those of loc. 3 in that the wings show a brown spot which is less expanded in the basal half of the wings, and in a less intensive colouration at the apex of the fore wings. This colouration is not similar to that of sympatric males, which have broad and almost completely blue wings, and it causes them to attract heterospecific males less frequently, hence they are likely to be less involved in heterospecific matings than heterochromics. This is probably the effect of their colouration, which is very different from that of *virgo* females. A wing colouration like that of homeochromics of loc. 3 would be unable to avoid reproductive interference because it mimics *haemorrhoidalis* males, which are constantly courted by *virgo* males.

In this way the extension of wing colouration in homeochromics seems to accord with that of the congeneric species found in the single sites and seems to be adaptive. Unfortunately in southern Italy only few sites are known where homeochromics are present, therefore a clear correlation between wing spot shape and sympatric species cannot be demonstrated.

Perhaps the recent discovery (OPPENHEIMER & WAAGE, 1987) of a method for obtaining *Calopteryx* matings under controlled conditions will make feasible *Calopteryx* rearing, and this will allow the study of the genetic basis of dimorphism, a necessity for a deeper comprehension of the phenomenon.

#### ACKNOWLEDGEMENTS

I am grateful to my friends Professor C. UTZERI, Dr G. CHIOZZI and Mr M. PAVESI and to an anonymous referee for helpful comments during the preparation of this paper.

#### REFERENCES

- BUCHHOLTZ, C., 1955. Eine vergleichende Ethologie der orientalischen Calopterygiden (Odonata) als Beitrag zu ihrer systematischen Deutung. *Z. Tierpsychol.* 12(3): 364-385.
- DUMONT, H.J., 1971. Need of protection of some European Odonata. *Biol. Conserv.* 3(3): 223-227.
- DUMONT, H.J., 1977. Sur une collection d'odonates de Yougoslavie, avec notes sur la faune des territoires adjacents de Roumanie et de Bulgarie. *Bull. Anns Soc. r. belge Ent.* 113: 187-209.
- FUDAKOWSKI, J., 1930. Über die Formen von *Calopteryx splendens* Harr. aus Dalmatien und Herzegovina (Odonata). *Anns Mus. zool. pol.* 1: 187-192.
- GRANT, P.R., 1972. Convergent and divergent character displacement. *Biol. J. Linn. Soc.* 4: 39-68.

- HEYMER, A., 1972. Comportements social et territorial des Calopterygidae (Odon. Zygoptera). *Annls Soc. ent. Fr.* (N.S.) 8(1): 3-53.
- HEYMER, A., 1973. Etude du comportement reproducteur et analyse des mécanismes déclencheurs innés (MDI) optiques chez les Calopterygidae (Odon. Zygoptera). *Annls Soc. ent. Fr.* (N.S.) 9(1): 219-255.
- HILTON, D.F.J., 1983. Mating isolation in two sympatric species of *Nehalennia* (Zygoptera: Coenagrionidae). *Odonatologica* 12(4): 375-379.
- HINNEKINT, B.O.N. & H.J. DUMONT, 1989. Multi-annual cycles in populations of *Ischnura e. elegans* induced by crowding and mediated by sexual aggression (Odonata: Coenagrionidae). *Entomol. gener.* 14(3/4): 161-166.
- JOHNSON, C., 1964. The inheritance of female dimorphism in the damselfly *Ischnura damula*. *Genetics* 49: 513-519.
- JOHNSON, C., 1966. Genetics of female dimorphism in *Ischnura demorsa*. *Heredity* 21: 453-459.
- JOHNSON, C., 1975. Polymorphism and natural selection in ischnuran damselflies. *Evol. Theory* 1: 81-90.
- MAIBACH, A., 1986. Révision systématique du genre *Calopteryx* Leach (Odonata, Zygoptera) pour l'Europe occidentale. 2. Analyses morphologiques et synthèse. *Mitt. schweiz. ent. Ges.* 59: 389-406.
- MAIBACH, A., 1987. Révision systématique du genre *Calopteryx* Leach pour l'Europe occidentale (Zygoptera: Calopterygidae). 3. Révision systématique, étude bibliographique, désignation des types et clé de détermination. *Odonatologica* 16(2): 145-174.
- MOORE, N.W., 1952. On the so-called "territories" of dragonflies (Odonata: Anisoptera). *Behaviour* 4: 85-100.
- OPPENHEIMER, S.D. & J.K. WAAGE, 1987. Handpairing: a new technique for obtaining copulations within and between *Calopteryx* species (Zygoptera: Calopterygidae). *Odonatologica* 16(3): 291-296.
- ROBERTSON, H.M., 1985. Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburi*: females mimicking males. *Anim. Behav.* 33: 805-809.
- ROBERTSON, H.M. & E.H. PATERSON, 1982. Mate recognition and mechanical isolation in *Enallagma damselflies* (Odonata: Coenagrionidae). *Evolution* 36(2): 243-250.
- RÜPPELL, G., 1985. Kinematic and behavioural aspects of flights of the male Banded Agrion, *Calopteryx (Agrion) splendens* L. In: M. Gewecke & G. Wendler, [Eds], *Insect locomotion*, pp. 195-204, Parey, Berlin-Hamburg.
- TENNESSEN, K.J., 1982. Review of the isolating barriers in Odonata. *Adv. Odonatol.* 1: 251-265.
- WAAGE, J.K., 1973. Reproductive behaviour and its relation to territoriality in *Calopteryx maculata* (Beauvois) (Odonata: Calopterygidae). *Behavior* 47(3-4): 240-256.
- WAAGE, J.K., 1975. Reproductive isolation and the potential for character displacement in the damselflies, *Calopteryx maculata* and *C. aequabilis* (Odonata: Calopterygidae). *Syst. Zool.* 24: 24-36.
- WAAGE, J.K., 1979. Reproductive character displacement in *Calopteryx* (Odonata: Calopterygidae). *Evolution* 33(1): 104-116.
- ZAHNER, R., 1960. Über die Bindung der Mitteleuropäischen *Calopteryx*-Arten (Odonata, Zygoptera) an den Lebensraum des strömenden Wassers. II. Der Anteil der Imagines an der Biotopbindung. *Int. Revue ges. Hydrobiol.* 45(1): 101-123.