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INDIVIDUAL RECOGNITION OF MATES AND NON-MATES BY MALE *CALOPTERYX SPLENDENS XANTHOSTOMA* (CHARPENTIER) (ZYGOPTERA: CALOPTERYGIDAE)

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The results of experiments are presented which suggest that males can discriminate between mates and non-mates. In trials with tethered females, males experienced an approximately 15 min 'refractory period' between mating once and remating. When given a choice between a recent mate, and a (recently mated) non-mate ovipositing on their territories, males were significantly more likely to mate a non-mate than a previous mate. The possible mechanisms for discrimination are considered.

INTRODUCTION

Individual recognition and discrimination, the processes whereby individuals assess and react to conspecifics on the basis of traits expressed by those individuals (WALDMAN et al., 1988) is most common in social insects, where it apparently amounts to a recognition of kin (but see GRAFEN, 1990). For example, nest mate recognition occurs in *Camponotus* ants (ERRARD & JALLON, 1987), honey bees *Apis*, (BREED, 1981; GETZ, 1991) as well as in primitively eusocial *Polistes* wasps (GAMBOA et al., 1986) and sweat bees *Lassioglossum* (MICHENER & SMITH, 1987). Amongst non-social insects, recognition operating to avoid inbreeding occurs in *Drosophila* (SPIESS, 1987). In these examples, the recognition signal is mediated chemically. It is unknown whether acoustically mediated individual mate recognition exists: there is some circumstantial evidence that it occurs in orthopterans, although male dominance status confuses the conclusions (GWYNNE, 1982; THORNHILL & ALCOCK, 1983). Individual recognition via visual cues is

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apparently unknown in insects and evidence for it in other invertebrates is equivocal (e.g. decapods, VANNINI & GHERARDI, 1981).

There is no reported instance of individual recognition in odonates, on the contrary; it has been stated that males of both European and North American calopterygids cannot distinguish mates from non-mates who are ovipositing on their territories (HEYMER, 1973; WAAGE, 1979a; ALCOCK, 1983). Here results of experiments are presented which suggest that males of the damselfly *Calopteryx splendens xanthostoma* (Charp.) can discriminate visually between mates and non-mates on their territories.

METHODS

Experiments were performed in July 1992 on the river Vidourle in southern France.

REFRACTORY PERIOD. – Males may be "programmed to experience reduced sexual motivation immediately after a copulation" (ALCOCK, 1983). To measure any such 'refractory period', 10 territorial males were presented with females tethered to a fishing rod with narrow gauge nylon monofilament (diameter 0.1 mm) looped around the thorax and abdomen, and tied dorsally to the 2nd thoracic segment (after SIVA-JOTHY & TSUBAKI, 1989). Tethered females had about 1.5 m of 'slack' monofilament and were able to fly, perch, copulate and oviposit normally. The time taken for the male to copulate with each of two females, presented consecutively, was measured. After the male copulated with the first female, she was removed and, two minutes later, a second unmated, 'novel' female was presented to the male for two minutes. This female was then removed and, after a further two minutes, presented again. This pattern was repeated. The trial was terminated either when the male copulated a second time, or after 24 minutes if no second copulation had occurred by then.

INDIVIDUAL RECOGNITION. – To test whether males can recognise individual females, two territorial males (not neighbours) were mated with tethered females. These experimental copulations were carried out within two minutes of each another. Two minutes after the end of copulation both females were introduced onto the territory of one of the males. Thus, this male had copulated with one of the presented females ('mate'), but was unfamiliar with the other (also recently copulated) female ('non-mate'). Both females were left on the territory for 1 minute in every 3 minutes, until the male copulated or 20 minutes elapsed. This procedure was repeated 15 times. Females were not marked in any way and each one was only used for one trial. Nine different territorial males were used.

RESULTS

REFRACTORY PERIOD

Males copulated on introduction of the first female after 17.9±3.0 s (n=10), but typically guarded a second, novel female for over 15 minutes before copulating a second time (985.2±271 s, n=10; this value includes the two minute periods when the female was absent from the territory). This difference is significant (t test, t=13.01, df=18, p=0.0001), suggesting that there is a constraint to remating. (The above means are given ± standard error).

INDIVIDUAL RECOGNITION

In 4 out of the 15 trials the territorial male had not copulated with either female after 20 minutes. These trials were abandoned. Of the remaining 11 trials, the territorial male copulated with the non-mate female nine times and the recent mate twice (82% recopulation with the novel female). Thus, given a choice between a female that he has just copulated with and a non-mate, males are more likely to copulate with the latter (50% binomial test, p=0.04 [one tail]).

DISCUSSION

C. s. xanthostoma males took significantly longer to copulate with a novel female presented at the territory when they had recently copulated with a different female as compared to when there had been no recent copulation.The period of sexual inactivity following a copulation ('refractory period') is probably the result of temporary sperm depletion (WAAGE, 1979a). ALCOCK (1983) suggests that the failure of males to copulate with a newly arriving non-mate may be due to a 'programmed' response of sexual inactivity immediately after copulation and, further, that it represents an inability of males to recognise their mates as individuals. However, given a simultaneous choice between a recent mate and a non-mate, males recopulated with the non-mate significantly more often than if they chose at random, implying that individual recognition does occur. In calopterygids stored sperm are removed from the female before new sperm are inseminated (WAAGE, 1979b; SIVA-JOTHY & TSUBAKI, 1989). Thus, there are obvious benefits to recognising previous mates because remating the same female means that a male removes his own sperm.

There are two, non-exclusive explanations for this observation, both based on individual recognition. Females may remember males with whom they have recently copulated and behave differently compared to females without experience of that male, or males may recognise and discriminate individual females.

Conceivably, females could remember the territory on which they copulated and behave differently compared to females without experience of that territory. However, there was no obvious difference in behaviour between the two females, mate and non-mate, during the experiment. Both appeared to oviposit normally, although it is possible that there was some subtle difference in behaviour that went unnoticed. Such a lack of behavioural differences between females suggests that the asymmetry in remating outcome is a result of discrimination by males between mates and non-mates. An individual-specific signal could be mechanically, chemically or visually transmitted. YACK & FULLARD (1993) review the occurrence of 'ears' in insects; there is no report of any such organ in odonates, nor have we any evidence for a behavioural response to sound in *C. s. xanthostoma.*

Furthermore, ovipositing females do not make any audible noise. Contact between two individuals is probably necessary for chemo-reception in odonates (Dr P.L. Miller, pers. comm.) and in the present trial this did not occur until the mating event itself.

Any recognition mechanism would almost certainly be visual. Vision in dragonflies is highly developed; they have colour vision extending into the ultra-violet and more than 80% of the brain is devoted to vision processing (ARMETT-KIBEL & MEINERTZHAGEN, 1983). Moreover, two recent papers suggest that visual processing in insects may be even more sophisticated than is supposed. SRINIVASAN et al. (1993) show that honey bees trained to recognise striped patterns do so by extracting features of the pattern (edges and bars) in a functionally analogous way to pattern analysis in vertebrates, and O'CARROLL (1993) has described classes of cells from the optic ganglion of the dragonfly *Hemicordulia tau*, which are functionally similar to those in the mammalian visual cortex, in that they are tuned to different size "target classes".

Individuals of *C. s. xanthostoma* have spatial memory: copulation often occurs in the bankside vegetation rather than on the male's territory, and at the end of copulation the female is released and the male immediately returns to his territory. After a short time (a few seconds) the female returns to her mate's territory even when she is not 'guided' by the male (personal observation). Similarly, males are 'territory faithful' and, after their overnight roost, will return to the same position on the river for several consecutive days. For males to discriminate individual females, an extension of the 'orientate by landmark' mechanism to one of finer resolution, is all that is required.

One possible cue used by males to distinguish individual females is the pseudopterostigma on the female's wings, a sexually dimorphic trait, and the only pigmented area on the female's wings. The number of cells of the pseudopterostigma which are pigmented varies between individuals. Female wings appear hyaline in the human visible spectrum, but there is good evidence for ultra-violet reflection on male and female dragonfly wings (e.g. HILTON, 1986); clearly this is a potential source of inter-female variation.

Many damselflies perform characteristic 'wing claps' whilst perching and ovipositing, i.e. slowly opening the wings then snapping them back together (WALKER, 1953). 'Wing claps' probably have a thermoregulatory and respiratory function (MILLER, 1994), although BICK & BICK (1978) suggested that this behaviour serves a communicative function in *C. maculata*. WAAGE (1984) describes a similar behaviour, 'wing flipping', which occurs in *C. maculata* and *C. dimidiata* as "an active display of receptivity by females". Wings vary in length between individuals, therefore the arc described by the pseudopterostigma during wing movements will be individual specific. Males may use any combination of the above variations to discriminate between individual females.

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REFERENCES

ALCOCK, J., 1983. Mate guarding and the acquisition of new mates in Calopteryx maculata (P. de Beauvois) (Zygoptera: Calopterygidae). *Odonatologica* 12: 153-159.

ARMETT-KIBEL, C. & A. MEINERTZHAGEN, 1983. Structural organization of the ommatidia in ventral compound eye of the dragonfly, Sympetrum. *J. comp. Physiol.* 151: 285-294.

BICK, G.H. & J.C. BICK, 1978. The significance of wingclapping in Zygoptera. *Odonatologica* 7: 5-9.

BREED, M.D., 1981. Individual recognition and learning of queen odors by worker honey bees. *Proc. natn. Acad. Sci. U.S.A.* 78: 2635-2637.

ERRARD, C. & J.M. JALLON, 1987. An investigation of the development of chemical factors in ants intrasociety recognition. *In*: J. Eder & H. Rembold [Eds], Chemistry and biology of social insects, pp. 478-498, Pepemy, München.

GAMBOA, G.J., H.K. REEVE & D.W. PFENNIG, 1986. The evolution and ontogeny of nestmate recognition in social wasps. *A. Rev. Ent.* 31: 431-454.

GETZ, W.M., 1991. The honey bee as a model kin recognition system. *In*: P.G. Hepper, [Ed.], Kin recognition, pp. 358-412, Cambridge Univ. Press, Cambridge.

GRAFEN, A., 1990. Do animals really recognise kin? *Anim. Behav.* 39: 42-54.

GWYNNE, D.T., 1982. Mate selection by female katydids (Orthoptera: Tettigoniidae, Concephalus nigropleurum). *Anim. Behav.* 30: 734-738.

HEYMER, A., 1973. Étude du comportement reproducteur et analyse des méchanismes déclencheurs innés (MDI) optiques chez les Calopterygidae (Odon. Zygoptera). *Annls Soc. ent. Fr.* (N.S.) 9: 219-255.

HILTON, D.F.J., 1986. A survey of some Odonata for ultraviolet patterns. *Odonatologica* 15: 335-345.

MICHENER, C.D. & B.H. SMITH, 1987. Kin recognition in primitively eusocial insects. *In*: D.J.C. Fletcher & C.D. Michener, [Eds], Kin recognition in animals, pp. 209-242, Wiley, New York.

MILLER, P.L., 1994. The functions of wingclapping in the Calopterygidae (Zygoptera). *Odonatologica* 23: 13-22.

O'CARROLL, D., 1993. Feature-detecting neurons in dragonflies. *Nature, Lond.* 362: 541-543.

SPIESS, E.B., 1987. Discrimination among prospective mates in Drosophila. *In*: D.J.C. Fletcher & C.D. Michener, [Eds], Kin recognition in animals, pp. 75-120, Wiley, New York.

SIVA-JOTHY, M.T. & Y. TSUBAKI, 1989. Variation in copulation duration in Mnais pruinosa pruinosa Selys (Odonata: Calopterygidae). *Behav. Ecol. Sociobiol.* 24: 39-45.

SRINIVASAN, M.V., S.W. ZHANG, & B. ROLFE, 1993. Is pattern vision in insects mediated by 'cortical' processing? *Nature, Lond.* 362: 539-540.

THORNHILL, R. & J. ALCOCK, 1983. *The evolution of insect mating systems.* Harvard Univ. Press, Cambridge, MA.

VANNINI, M. & F. GHERARDI, 1981. Dominance and individual recognition in Potamon fluviatile (Decapoda, Brachyura)-possible role of visual cues. *Mar. Biol. Physiol.* 8: 13-20.

WALDMAN, B., P.C. FRUMHOFF & P.W. SHERMAN, 1988. Problems of kin recognition. *TREE* 3: 8-13.

WAAGE, J.K., 1979a. Adaptive significance of postcopulatory guarding of mates and non-mates by male Calopteryx maculata (Odonata). *Behav. Ecol. Sociobiol.* 6: 147-154.

WAAGE, J.K., 1979b. Dual function of the damselfy penis: sperm removal and transfer. *Science* 203: 916-918.

WAAGE, J.K., 1984. Female and male interactions during courtship in Calopteryx maculata and C. dimidiata (Odonata: Calopterygidae): influence of oviposition behaviour. *Anim. Behav.* 32:

400-404.
WALKER, E.M., 1953. *The Odonata of Canada and Alaska*, Vol. 1. Univ. Toronto Press, Toronto.
YACK, J.E. & J.H. FULLARD, 1993. What is an insect ear? *Ann. ent. Soc. Am.* 86: 677-682.