

Discriminative mate choice in relation with female maturation in *Ischnura elegans* (Odonata: Coenagrionidae)

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Abstract

It is often assumed that males do not choose among females because competition for partners is high and male mating costs are low. Nevertheless, this assumption does not always hold. In some species duration of copulation is long, possibly causing a limitation on the lifetime number of matings for males. In this case we expect males to discriminate among females differing in quality. We first discuss quality differences between immature and mature females. Second, we test whether males of the zygopteran *Ischnura elegans* discriminate between immature and mature females. The hypothesis was examined by performing binary choice experiments in small cages in three different populations. To examine possible mechanisms for discrimination we excluded behavioural differences in one of the two experiments by using dead females. The results show that males of *I. elegans* prefer mature to immature females and that this choice is probably based upon a difference in body coloration.

Introduction

To achieve successful matings, interspecific discrimination and also discrimination among conspecific individuals is required. The latter can be defined as mate choice, i.e. any pattern of behaviour shown by the members of one sex that leads to them being more likely to mate with certain members of the opposite sex than with others (Halliday 1983). Mate choice requires some sort of signalling on which mating decisions can be based. Such signals can be visual, auditory and/or chemical (Andersson 1994). While most recent work on sexual selection by mate choice has emphasised female mate choice (reviewed by Kirkpatrick and Ryan 1991), relatively few studies have examined male mate choice. Males are assumed not to discriminate due to strongly male-biased operational sex ratios in insect populations (e.g. Nylin et al. 1995;

Godfray & Werren 1996). If males outnumber females, it is non-adaptive for males to skip mating opportunities because, due to intrasexual competition, the probability of achieving a future mating might be low (e.g. Kvarnemo & Ahnesjö 1996). In addition, it is often assumed that males are indiscriminate in selecting partners because copulations are not costly for them (e.g. Thornhill & Alcock 1983; Alcock 1989).

However, the latter is not always correct (e.g. Wiernasz 1995). First, the risk of predation may increase for males engaged in mating activities, mainly owing to their breeding coloration and/or conspicuous courtship displays, mate searching and assessment, or mating itself (reviewed by Lima & Dill 1990; Magnhagen 1991). Secondly, in some animals the number of matings a male can achieve is limited due to energetic and time constraints (e.g. Anholt et al. 1991; Anholt 1992; Blanckenhorn et al. 1995). If the number of copulations is limited, we expect males to exercise mate choice in favour of high quality females (e.g. Dewsbury 1982).

Probably the most extreme quality difference is between immature and mature females. Immature individuals are defined as sexually immature but post-teneral. While mature females normally carry a clutch of eggs, immature ones do not and therefore have a lower immediate fitness value for males. Indeed, immature females have to survive until maturity before they can lay fertile eggs. Moreover, in species where females mate several times, we have to consider sperm competition. If last-male sperm precedence is important, to inseminate an immature female would mean taking the supplementary risk that another male would remove that sperm before the female reaches maturity. In both cases the lifetime mating success of a male mating with an immature female might be strongly reduced. Nevertheless, in low-density populations males may still be indiscriminate, because of the risk of not obtaining any copulation at all.

In this study we look for the existence of male discrimination between immature and mature females in Zygoptera. Some females of *Ischnura* species seem to mate only once during their life time (Fincke 1987; Robinson & Allgeyer 1996), others in an immature stage or even directly after emergence (Rowe 1978; Hinnekint & Dumont 1989), whereas other species mate several times (e.g. Robertson 1985; Cordero et al. 1998). Mating behaviour of many male Zygoptera seems to be rather indiscriminate, even resulting in male-male tandems and triple connections (Corbet 1999)

This suggests that males are attracted to any Zygoptera. However, since in the genus *Ischnura* copulation can last for several hours (Krieger & Krieger-Loibl 1958; Robertson 1985; Miller 1987; Cordero 1990) this activity can be expected to have high mating costs. Furthermore because these Zygoptera also have a short lifespan (e.g. Parr & Parr 1972; Van Noordwijk 1978), their lifetime mating success is accordingly reduced. In contrast to most other Zygoptera, many immature *Ischnura elegans* (Vander Linden) do not leave the waterside during sexual maturation (Parr 1973). Females mate several times (Cordero et al. 1997; Van Gossum et al. 1999a) and the most recent male copulation partner sires most offspring (Cooper et al. 1996). Therefore, it is expected to be highly adaptive for a male to discriminate between immature and mature *I. elegans*.

A difficulty in most studies is that male choice is often examined by presenting only one model per trial (e.g. Cordero 1989; Gorb 1999). This may however confound

male choice and willingness to mate. To avoid this, we performed binary choice experiments (see also Van Gossum et al. 1999b). The aims of this study are to examine if mature males of *I. elegans* discriminate between immature and mature conspecific females and if so, on which mechanism (i.e. behaviour and/or morphology) they base their discrimination.

Methods

In *Ischnura elegans* several different female colour morphs coexist. We will apply the names for female colour morphs as suggested by Parr (1999): andromorph and gynomorph. The colour polymorphism is partly age-related, there being two immature and three mature female colour morphs (Parr 1965). The two immature female morphs, *f. rufescens* and *f. violacea*, have respectively an orange and a violet meso- and metathorax. The short pale green stage following the *violacea* stage is not considered in this study. Mature females have a turquoise to blue (one morph, andromorph females) or a brownish (two morphs, gynomorph females) meso- and metathorax (Killington 1924; Parr 1965). The differences between the two brownish female morphs are only minor and are restricted to the absence of black humeral stripes on the thorax of one of the morphs, while the pale parts of the thorax also differ slightly in colour. Because previous experiments (Van Gossum et al. 1999a) indicated that males do not distinguish between the two brownish or gynomorph females, both were grouped in this study. The species is one of the most common Zygoptera in west Europe (Askew 1988).

To test male choice between immature and mature females, a binary choice experiment was carried out in small cages during the summer of 1996. Cages (30x30x30 cm) consisted of a metal frame and bottom. The frame was covered with a net with small mesh size (1x1 mm). We used animals from three populations in northern Belgium: Neerhelst (51°11'N, 4°56'E) 15 and 16 August 1996, Den Diel (51°15'N, 5°12'E) 18 and 19 August 1996, and De Maat (51°14'N 5°12'E) 20 and 21 August 1996. In these three localities we investigated, respectively, ca 60, 80 and 40 m of shoreline. Population densities were rather low, there being a maximum of approximately three males per meter of shoreline (for a comparison see Hinnekint 1987). All three study populations were strongly male-biased (Table 1). The distances between sites were ca 2-30 km. All captured animals received a mark in black ink on the left forewing (Staedtler Pancolor, 0.3 mm pen). At each study site we captured and marked as many of the population as possible. After marking, mature males were stored in empty film canisters (black) to prevent habituation to colour during the period between capture and the choice experiment. We placed one mature and one immature female morph in the cage at the population site before a single mature male (blue-green or blue thorax) was introduced. The trial was finished when a male made a selection or after a time span of 120 s. Then males and females were replaced. We interpreted a male attempt to grasp a female as an indication of mate choice. Males that did not try to grasp a female were excluded from the analysis. The experiment was performed at least eight times in each population for the combinations *rufescens* versus andromorph female,

and *rufescens* versus gynomorph female. The combinations between *violacea* and the two mature female morphs were only examined in two populations (Den Diel and De Maat), due to low numbers present at the time of the study.

Because in the first experiment males could discriminate between immature and mature females on the basis of behaviour and morphological characteristics, we set up a second experiment excluding behaviour. Any discrimination in this second experiment would indicate that behavioural components are less important for mate choice between immature and mature females. Therefore, we analysed male mate choice by presenting combinations of dead tethered females (*rufescens* versus mature female) (14, 16 and 22 August 1996). Animals were collected at S-pond located in Wilrijk, a low-density population. Two freshly killed females were pinned (roughly 5 cm apart) on a thin branch. We used two insect pins for each female, one to pin the thorax, while the other was used to position the abdomen straight backwards. One of us walked slowly around in an insectary (3x3x2.5 m; see Van Gossum et al. 1999a for description) while moving the branch with the pinned females up and down, in front of free-flying males. Mate choice was scored as in experiment one.

We first examined differences of occurrence in the population between immature and mature females in the three populations, performing binomial tests. This was done to avoid the possibility that our findings were only the result of males preferring the most abundant morph in the population (Fincke 1994; Van Gossum et al. 1999b). Male choice between *rufescens* and mature females in the three populations was analysed by performing a log linear analysis on the frequencies with STATISTICA 5.0 (Statsoft 1994). Population was included as a factor in the analysis to assess whether male choice was consistent among sites. Both the preference experiment between *violacea* and mature females, and the second experiment with dead females were analysed using binomial tests.

Results

No difference was found between the frequencies of immature and mature females in the three populations (Table 1; binomial tests: Neerhelst: $p = 0.71$, Den Diel: $p = 0.73$, De Maat: $p = 0.86$). Mature females were preferred over the immature colour morph *rufescens* in all three study plots (Fig. 1; log linear analysis, age: $\chi^2 = 26.95$, $df = 1$, $p < 0.001$). This preference did not differ among populations (log linear analysis, population x age: $\chi^2 = 0.83$, $df = 2$, $p = 0.65$). There was a strong tendency that gynomorph females were preferred over *violacea* in Den Diel (binomial test; $p = 0.055$) and andromorph females over *violacea* in De Maat ($p = 0.062$). Overall, in Neerhelst males preferred mature females in 84 % ($n = 19$), in Den Diel in 85 % ($n = 26$) and in De Maat in 83 % ($n = 30$) of the cases (Fig. 1).

In the second choice experiment with dead females, males preferred mature females over immature females (*rufescens*) in 83 % of the cases (binomial test; $p = 0.004$). Male preference for mature females did not differ between experiments with live and dead females ($\chi^2 = 0.085$, $df = 1$, $p = 1.00$).

Table 1. Total numbers (sum of two sampling days) of immature and mature females, and mature males of *Ischnura elegans* in three study populations.

	Neerhelst	Den Diel	De Maat
Immature females	13	15	17
Mature females	16	18	15
Males	70	95	104
Metres of shoreline sampled	60	80	40
Male density (per m shoreline)	1.17	1.19	2.60
Sex ratio (males / females)	2.41	2.88	3.25

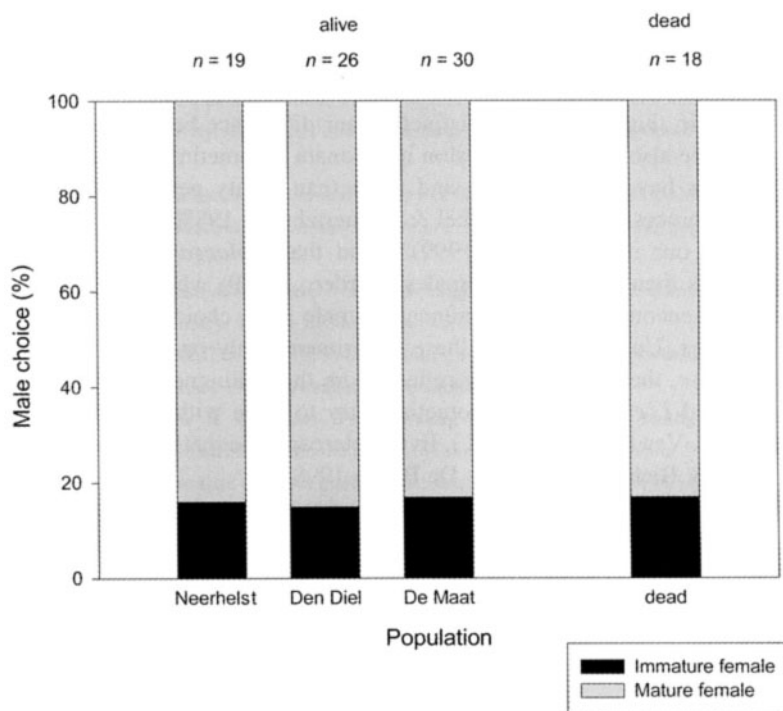


Figure 1. Male preference for live and dead immature versus mature females in *Ischnura elegans* (n gives the number of trials).

Discussion

In this study we found clear evidence that males of *Ischnura elegans* prefer to mate with mature females given the choice between immature and mature females. Recent theoretical studies indicated that high levels of competition for mates, as well as mate choice, may arise in the same sex if variation in mate quality is high and the number of matings is limited (Owens & Thompson 1994; Owens et al. 1994; Andersson & Iwasa 1996; Johnstone et al. 1996). These assumptions are fulfilled for males in our study species. First, there is a great immediate difference in quality between immature and mature females. Second, the short life span, the long copulation duration and the male-biased sex ratio at the mating site are all factors limiting the number of matings (Parr & Palmer 1971; Hinnekint 1987).

Since the choice pattern of males is not different in experiments with living and with dead females, we believe that the most likely reasons to explain choice in this study are the distinct colour differences between immature and mature females. This does not exclude the possibility that males also may discriminate on the basis of female behaviour (e.g. Fincke 1987). We suggest, however, that morphological characteristics may be more important for males to distinguish between immature and mature females. In the zygoptera studied, external morphology, especially coloration of the wing or thorax, are in general most important for visual recognition of sex by males (for a review see Corbet 1999). Therefore, we believe that the best candidate to explain choice in this study is the distinct colour difference between immature and mature females (see also Parr 1965). Vision in Odonata is sometimes highly developed: males and females have colour vision and more than eighty percent of the brain is devoted to vision processing (Amett-Kibel & Meinertzhagen 1983).

In contrast to our results, Gorb (1999) found that *violacea* females were more preferred by males than gynomorph females. Cordero (1989) who studied *I. graellsii* (Rambur) did not encounter clear differences in male mate choice between immature and mature females. Unfortunately, in these experiments only one morph was offered at a time. Therefore, their findings may reflect more the willingness of males to mate. Indeed, in the field *I. elegans* males sometimes try to mate with immature and even teneral animals (H. Van Gossum unpubl.). Even heterospecific matings sometimes occur in the field (Bick & Bick 1981; Stoks & De Bruyn 1998).

We do not know if it is possible to extrapolate our results to other species. In most Zygoptera, immature animals only return to the mating site after the maturation period (e.g. Miller 1987). In these cases males do not have to discriminate between immature and mature females. Therefore we predict that discrimination is only needed in species where immature and mature animals coexist, as in *I. elegans*.

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