

**MALE HARASSMENT ON FEMALE COLOUR MORPHS
IN *ISCHNURA ELEGANS* (VANDER LINDEN):
TESTING TWO FREQUENCY-DEPENDENT HYPOTHESES
(ZYGOPTERA: COENAGRIONIDAE)**

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In many Zygoptera spp. ♀♀ occur in different colour morphs, with one morph coloured like the ♂ (andromorph), while the others are not (gynomorph). Two hypotheses have been proposed to explain frequency-dependent harassment of ♀ morphs. According to the first, ♂♂ should prefer the more frequent of the 2 ♀ morphs (learned-mate recognition hypothesis). According to the second, ♂♂ should prefer andromorphs more if their frequency relative to ♂♂ increases, but not so for gynomorphs which always should be attempted to mate with on encounter (mimicry hypothesis). Here, it is reported on a re-analysis of earlier published data on morph-specific harassment for *I. elegans*, which allows examination of the 2 proposed hypotheses. The data were collected in 8 insectaries with different ratios of ♂♂ and ♀ morphs. As reported earlier, ♂ harassment is highest on the most common ♀ morph supporting the learned-mate recognition hypothesis. The ratio of andromorphs to ♂♂ had no morph-specific effects in amounts of ♂ harassment, wherefore the data suggest rejection of the mimicry hypothesis.

INTRODUCTION

Coexistence of multiple female colour morphs within a species is a characteristic of many damselflies and dragonflies (e.g. CORBET, 1999; FINCKE, 2004). Typically, one of the female damselfly morphs (andromorph) is coloured, and in some species also patterned like the male, while the other morphs (gynomorphs)

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are different (e.g. CORBET, 1999). Despite understanding of the mode of inheritance in several damselfly species (JOHNSON, 1964, 1966; CORDERO 1990; ANDRÉS & CORDERO, 1999), and molecular and experimental support for the polymorphism to be under selection (ANDRÉS et al., 2000, 2002; SIROT & BROCKMANN, 2001; WONG et al., 2003), considerable debate remains about the underlying mechanisms involved in the maintenance of multiple female colour morphs (e.g. FINCKE, 1994; CORDERO et al., 1998; SHERRATT, 2001; VAN GOSSUM et al., 2001a, 2001b; SIROT et al., 2003). A mathematical model (SHERRATT, 2001), a verbal model (FINCKE, 2004) and empirical studies (e.g. MILLER & FINCKE, 1999; VAN GOSSUM et al., 2001b) indicate that frequency-dependent sexual harassment by males may contribute to the maintenance of multiple female colour morphs. However, the exact nature of the frequency-dependent selection is being debated and two hypotheses have been proposed.

According to one view, males will prefer to mate with the most common female morph in their population and will change this mate preference according to female morph frequency (ratio of andro- to gynomorphs); i.e. the learned-mate recognition hypothesis or LMR (MILLER & FINCKE, 1999). FINCKE (2004) recently elaborated on her idea and suggests conspecific males, or even heterospecific males and females also may affect a male's capability of partner selection. In short, the amount of a male harassment toward a female morph may depend on the ratio of this morph to the number of distractors present in a population (FINCKE, 2004). These may differ between populations because, for example, phenotypically similar looking heterospecific individuals may not be present in all populations of a species. In support of the LMR several studies indeed showed that the ratio of andro- to gynomorphs in a population was a good predictor of male mate choice for female colour morphs (MILLER & FINCKE, 1999; VAN GOSSUM et al., 1999; VAN GOSSUM et al., 2001a, 2001b).

Second, according other researchers, andromorphs are considered to be functionally male mimics with males being incapable of distinguishing between these females and other males at least under some conditions (e.g. ROBERTSON, 1985; HINNEKINT, 1987). In this context SHERRATT (2001) developed a mathematical model to explain the maintenance of multiple female colour morphs. SHERRATT (2001) assumes that the frequency of harassment on andromorphs is positively correlated with the ratio of andromorphs to males (in this case males could be considered distractors) in the population (the so-called mimicry hypothesis). Hence, a male's ability to recognize a female depends on how many andromorphs and conspecific males are present in a population. For gynomorphs such a relationship is not to be expected and this morph should always be mated with on encounter (SHERRATT 2001).

Under some circumstances the LMR and the mimicry hypotheses may give identical predictions (see further), wherefore prior studies indicating support for the LMR must be questioned. Can the mathematical model by SHERRATT (2001)

explain the currently published empirical data in the literature? Today, one of the best tests of the LMR remains the study by VAN GOSSUM et al. (2001a). They first tested males from the field in simple binary choice tests. After these tests, the males, which had attempted to mate with andromorphs, were placed together in an insectary along with twenty gynomorphs, while those males, which had attempted to mate with gynomorphs, were placed together, along with twenty andromorphs. The authors viewed this as a period in which males were likely to switch their mate choice based on the ratio of the female morphs. However, it is important to note that by caging males together with females the ratio of andromorphs to males was also radically altered (Thomas Sherratt, pers. comm.). If males are kept with gynomorphs, then it is reasonable to suggest that males might begin to learn that anything male-like was unprofitable. Thus, the preference for gynomorphs, following a period in an insectary with only gynomorphs and males might well be because the significant majority of males simply learn to avoid mating with anything male-like, rather than learn to recognize gynomorphs as potential mates. The reported preference of males for andromorphs may arise as a consequence of the perceived ratio of andromorphs to males as predicted by Sherratt's model. To conclude, previous work is consistent with both hypotheses, so cannot discriminate between them (T. Sherratt, pers. comm.). Here, we re-analysed the data of an earlier published study on the frequency of male harassment on the two female colour morphs of *I. elegans* (VAN GOSSUM et al., 2001b). While in the original study morph-specific degrees of harassment were only examined in the light of variation in the ratio of andro- to gynomorphs (LMR), here we also explore the effects of variation in the ratio of andromorphs to males (mimicry hypothesis).

METHODS

To distinguish between the LMR and the mimicry hypotheses we re-analysed the data of VAN GOSSUM et al. (2001b). The experiment manipulated morph frequencies, densities and sex ratio in insectaries to evaluate their effects on the amount of male harassment on andro- and gynomorphs. Since no other damselfly species were present in the experimental cages, the distractor set for the males reduces to the two female morphs and other males (cf. FINCKE, 2004). Insectaries were filled with not yet sexually active, field-caught animals randomly distributed among eight outdoor insectaries (3×3×2.5 m). Insectaries contained small ponds, sufficient food and suitable vegetation.

Table 1
Ratio of andro- (A) to gynomorph (G) females and ratio of andromorphs (A) to males in the eight insectaries examined for this study

Ratio	Insectary							
	1	2	3	4	5	6	7	8
A- to G-females	0.25	0.25	0.25	0.25	1.5	4.0	4.0	4.0
A-females to males	0.1	0.2	0.4	0.4	1.0	0.4	0.4	1.6

In contrast to the situation in many other damselfly species, ratios of andro- to gynomorphs are highly variable in natural populations of *I. elegans*, with some populations dominated by gynomorphs and others dominated by andromorphs (PARR, 1965; VAN GOSSUM et al., 1999; SÁNCHEZ-GUILLÉN et al., In press). Therefore, ratios of andro- to gynomorphs applied in the study by VAN GOSSUM et al. (2001b) fell within the range observed in nature. Across insectaries, the ratio of andro- to gynomorphs (ranging from 0.25 to 4) and the ratio of andromorphs to males (ranging from 0.1 to 1.6) (Tab. I) were uncorrelated ($N = 8$, $r^2 = 0.58$, $p = 0.135$). Population density in the insectaries ranged from 15 to 30 individuals and sex ratio were female- or male-biased. Densities were in accordance with density estimations from natural populations (MOORE, 1964; HINNEKINT, 1987).

Maturation in *I. elegans* takes on average six days (CORDERO et al., 1998). The experiment started when animals were 15 days old, assuring they were all sexually mature. Male approaches towards females were recorded daily from 8h00 until 18h00 Mid European Summer Time on three consecutive days (5, 6 and 7 August). During each of these days observation sessions of fifteen minutes were rotated among insectaries, resulting in the monitoring of all the insectaries every two hours. The rotation sequence was randomly determined at each sampling date. Observations on male harassment were made from outside the insectaries with the naked eye and by using binoculars (Opticron, 10×42). First focus was on male approach and only second on the approached female morph, ensuring that the observed female encounter frequencies are unbiased by morph.

To maintain constant densities and frequencies of males and female colour morphs, each time an individual died it was replaced by a dummy. A dummy was a new, living damselfly identical in morph/sex to the animal that died, and was free to interact with the other individuals in the respective insectaries. During the three consecutive days five damselflies died and were replaced with newly caught sexually mature damselflies. Three of these were males, which adapt very fast behaviourally to new morph frequencies (MILLER & FINCKE 1999; VAN GOSSUM et al. 2001a; MILLER & FINCKE, 2004). The two other animals were andromorphs in insectary eight, an insectary with sixteen andromorphs. Even if the dummy females would have behaved differently, it is therefore highly unlikely that they biased our results.

Differences in male harassment between female colour morphs were analysed with mixed model regression (SAS 8.0, proc MIXED; LITTELL et al., 1996). The amount of male harassment was \log_{10} transformed in order to meet the assumptions of the statistical analysis. We used insectaries as our sample units and averaged the frequency of male harassment on each female morph per insectary per day. This resulted in coupled scores of both morphs for each insectary. We took this into account by nesting morphs within insectaries. Because for each insectary the same animals (with five exceptions, see above) were involved during all three days, we considered successive daily observations of male harassment in each insectary as repeats. Colour morph, and respectively the ratio of andro- to gynomorphs or the ratio of andromorphs to males were treated as independent, fixed effects. A backward stepwise model selection was applied in order to obtain the final model (CRAWLEY, 1993). The degrees of freedom of the fixed effects F-tests were adjusted for statistical dependence using the Kenward-Roger formula (KENWARD & ROGER, 1997; SCHAALJE et al., 2001).

RESULTS

Examination of the LMR indicated a significant interaction between colour morph and ratio of andro- to gynomorph females influencing the amount of male harassment on female morphs ($F_{1,22} = 5.02$, $p = 0.036$) (Fig. 1). When the ratio andromorph/gynomorph increases, the number of male mating attempts on andromorphs increases [$\log_{10}(\text{mating attempts}) = 0.010 + 0.052 \text{ A/G ratio}$] and decreases for gynomorphs [$\log_{10}(\text{mating attempts}) = 0.180 - 0.017 \text{ A/G ratio}$]. The less frequent morph was always subjected to fewer mating attempts compared to the

most common female morph (Fig. 1). Exploring whether the ratio of andromorphs to males (mimicry hypothesis) influences the amount of male harassment on female morphs indicated no morph-specific effects ($F_{1,22} = 0.08$, $p = 0.782$). A slight tendency was revealed that females were harassed less when there were more andromorphs relative to conspecific males ($F_{1,22} = 3.95$, $p = 0.060$), but such changes in amount of harassment were identical for andro- and gynomorphs.

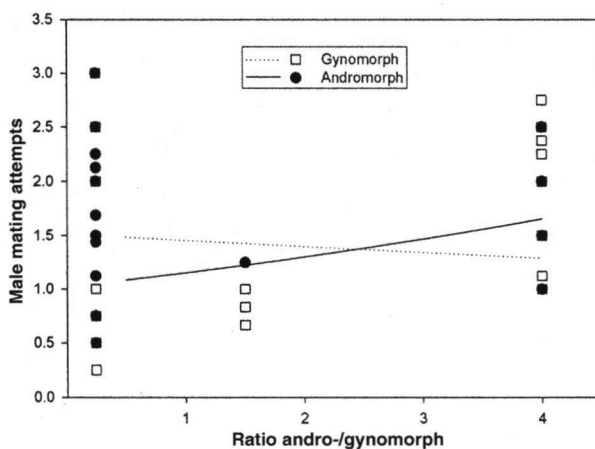


Fig. 1. Effect of ratio of andro- to gynomorph females on the frequency of morph-specific male harassment (male mating attempts). Dots represent the actual observed data averaged per female morph per day. Total number of dots is 24 for each female morph (8 insectaries with observations repeated for 3 days).

DISCUSSION

Our results did support the LMR. In concordance with earlier findings on *Enallagma* and *Ischnura* (MILLER & FINCKE, 1999; VAN GOSSUM et al., 2001a, 2001b) andromorphs were subject to more harassment under all densities and sex ratios if they constituted the majority female morph, but received least harassment when gynomorphs were more common. Results from field studies indicate that males are more attracted to gynomorphs (e.g. ROBERTSON, 1985; FINCKE, 1994), which are usually the commonest morph (McKEE et al., 2005). Furthermore, FORBES (1994) showed a preference for andromorphs when they were the naturally occurring majority morph.

Interestingly, SHERRATT (2001) assumed that gynomorphs are not expected to be harassed in a frequency-dependent way, i.e. should receive a uniform amount of harassment irrespective of their frequency if compared to andromorphs or if compared to males. Indeed, based on the observation that gynomorphs are not mimics any male in search of a female that encounters a gynomorph should always attempt to mate with her (SHERRATT, 2001). As a consequence, andromorphs are never expected to receive more male harassment than gynomorphs. Nonetheless, our results indicate that andromorphs do receive more male harassment if they are the more common female morph compared to gynomorphs.

Further, our results show that the amount of male harassment did only slightly depend on the ratio of andromorphs to males. Interestingly, male harassment on females decreased slightly when the ratio of andromorphs to males increased, and this so identical for andro- and gynomorphs. This is in contrary to expectations under the mimicry hypothesis (SHERRATT, 2001) where gynomorphs are expected to have equal amounts of harassment under all frequencies and andromorphs to receive more harassment than gynomorphs when they are relatively common compared to males. Currently, there is only one other study that can be used to evaluate the mimicry hypothesis. In an experiment conducted in captivity with *Ischnura ramburi* (Sel.), andromorphs that were confined to a cage at high operational sex ratio (one andromorph and three males) mated significantly more than those in a cage at low operational sex ratio (two andromorphs and two males) (SIROT & BROCKMANN, 2001). Gynomorphs showed no difference in mating frequency under the different treatments (SIROT & BROCKMANN, 2001). In that case, the results for gynomorphs were consistent with the predictions under the model by Sherratt, but the results for andromorphs were opposite to those predicted under the model. Nonetheless, the authors argued that since the damselflies in this experiment were confined to small enclosures potentially the male mimicry of andromorphs was broken by their inability to get away from males (SIROT et al., 2003). We believe that gynomorph behaviour in the small cages likely also differed from the natural situation. Indeed, in nature andromorphs use more open habitat and do not fly large distances, but gynomorphs hide more in the vegetation and fly longer distances (VAN GOSSUM et al., 2001b). Therefore, we consider it more likely that the results for gynomorphs in the small cages deviate from the situation in a natural population. Lastly, in most damselfly populations operational sex ratios are strongly male-biased (e.g. CORBET, 1999). Therefore, males will outnumber andromorphs by far at the mating site, indicating that differences in andromorph to male ratios will be very small and might be hard to detect (at least by human observers). In contrast, relative frequencies of both female morphs may be much easier to detect and used as a proximate cue for differential male harassment.

Together, we think that the mimicry hypothesis (SHERRATT, 2001) cannot provide explanation for the empirical findings on the studied *Ischnura* species. Following the results of our re-analysis neither the harassment patterns for andromorphs, nor those for gynomorphs do support the expectations under the mimicry hypothesis. The learned-mate recognition hypothesis, on the contrary is fully supported, and appears most consistent with all current field and experimental work (see also FINCKE, 2004).

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