Evol Ecol (2011) 25:139–154
DOI 10.1007/s10682-010-9388-z

RESEARCH ARTICLE

Reflectance spectra and mating patterns support intraspecific mimicry in the colour polymorphic damselfly *Ischnura elegans*

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Received: 17 August 2009 / Accepted: 29 April 2010 / Published online: 19 May 2010
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Abstract Coexistence of female colour morphs in animal populations is often considered the result of sexual conflict, where polymorphic females benefit from reduced male sexual harassment. Mate-searching males easily detect suitable partners when only one type of female is present, but become challenged when multiple female morphs coexist, which may result in frequency-dependent mate preferences. Intriguingly, in damselflies, one female morph often closely resembles the conspecific male in body coloration, which has lead to hypotheses regarding intra-specific male-mimicry. However, few studies have quantitatively evaluated the correspondence between colour reflectance spectra from males and male-like females, relying instead on qualitative visual assessments of coloration. Using colour analyses of reflectance spectra, we compared characteristics of the body coloration of ontogenetic male and female colour morphs of the damselfly *Ischnura elegans*. In addition, we evaluated whether males appear to (1) discriminate between immature and mature female colour morphs, and (2) whether male-like females experience reduced male mating attention and low mating frequencies as predicted from

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male-mimicry. Spectral reflectance data show that immature female morphs differ substantially in coloration from mature individuals. Mating frequencies were much lower for immature than mature female morphs. For the male-like female morph, measures of colour were statistically indistinguishable from that of both immature and mature conspecific males. Mating frequencies of male-like females were lower than those of other mature female morphs under field and experimental conditions. Together, our results indicate that males may use the observed spectral differences in mate choice decisions. Furthermore, male-like females may be regarded as functional mimics that have reduced attractiveness and lowered rates of sexual harassment by mate-searching males.

Keywords Intrasexual colour polymorphism · Mate searching · Sexual conflict · Signalling

Introduction

Colour variation between members of the same species where individuals can be categorized into different colour morphs is a common and often striking phenomenon found in most animal groups, with well-known examples in cichlid fish, geese, skuas, guppies and moths (Houde 1997; Seehausen et al. 1999; Bond and Kamil 2002; Mundy et al. 2004). In some cases, such colour polymorphism is restricted to one sex only, leading to the suggestion that this phenomena has evolved in the context of sexual selection (e.g. Gross 1996). For example, the occurrence of male-limited polymorphism in some species has been explained as a consequence of male-male competition over mates (reviewed in Oliveira et al. 2008). Female-limited polymorphism also occurs in a variety of animal taxa, but has received less attention, despite being more common than male-limited polymorphisms in some taxa (e.g., damselflies, Van Gossum et al. 2008). However, interest in this phenomenon has increased in the last few years as an arena for testing the role of sexual conflict in the evolution and maintenance of sex-limited polymorphisms (e.g. Hardling and Bergsten 2006; Svensson et al. 2009).

Sex-limited polymorphisms may occur as an evolutionary response to sexual conflict over mating frequency. Indeed, males will often pursue a higher mating frequency than females, a disparity between the sexes that can result in a conflict between eager males and selective females over the optimal mating frequency (e.g. Arnqvist and Rowe 2005). In fact, repeated sexual harassment by males may upset female time and energy budgets (*cf.* Bots et al. 2009), while potentially increasing the likelihood of physical damage (e.g. Jormalainen et al. 2001). Ultimately, such detrimental influences are expected to decrease female realized fecundity (Plath et al. 2003; Gosden and Svensson 2007). Selection should therefore favour female traits which reduce male mating interests to levels that correspond more closely to female optimal mating frequency. The evolution of multiple female colour morphs is thought to be one solution for reducing excessive male attention by interfering with the mechanisms males use to visually recognize potential mates.

In damselflies (Odonata: Zygoptera), female colour polymorphism is considered to be such a case (reviewed in Van Gossum et al. 2008; Svensson et al. 2009). Current evidence suggests that male damselflies may face little difficulty in detecting suitable mates when only one female type is present, but become challenged when multiple female types coexist at the reproductive site (e.g. Fincke 2004). More specifically, males are thought to adopt positive frequency-dependent mating preferences when faced with multiple female morphs (e.g. Miller and Fincke 1999; Van Gossum et al. 2001a). Similar to frequency-dependent predation, where predators predominantly attack more common forms of prey in an environment (e.g. Bond and Kamil 2002), mate-searching males are expected to more readily associate the visual characteristics of the most common female morph with potential mating opportunities. One exception to the expectation of strict frequency-dependent male responses in the presence of multiple female morphs arises when one female morph resembles an existing phenotype that is unprofitable in the context of mating (e.g., a male phenotype). These females should experience consistently lower levels of male harassment because male responses to these colour phenotypes should be under countervailing selection. It is intriguing in this context that in many damselfly species one female morph (the andromorph) resembles (at least to the human eye) the male colour phenotype, while the other (the gynomorph) exhibits coloration distinct from the male phenotype (e.g. Fincke et al. 2005). This observation has lead to the suggestion that andromorph females are functional male-mimics and consequently show reduced mating frequencies (Robertson 1985; Sherratt 2001).

Considering that odonates have well developed colour vision (Briscoe and Chittka 2001), female morph differences in body colour may be particularly relevant for male mate recognition and mating decisions. However, most previous research quantifying colour variation in damselflies has relied on assessment via human vision and/or methods generally criticized for human-related biases (e.g. the use of digital photography, Joop et al. 2006; but see Fincke et al. 2007; Contreras-Garduno et al. 2008; Schultz et al. 2008). In fact, only one previous study used appropriate, contemporary techniques for studying colour variation between males and colour polymorphic female damselflies. It was shown for two species of the genus *Enallagma* (where male-like females resemble males in body colour but not in pattern), that andromorphs have similar, but not identical, reflectance spectra as males (Fincke et al. 2007). Variation in the correspondence of a mimic to its model is common in nature (e.g. Edmunds 2000), and such imperfect mimicry is often considered a successful strategy in the context of aposematism (Sherratt 2002). For example, a hoverfly does not need to resemble a wasp perfectly to achieve protection against predators (e.g. Edmunds 2000). We are unaware, however, of any studies examining colour variation in species where andromorphs exhibit more precise resemblance of the male phenotype.

For most polymorphic species of damselflies, one male phenotype and two mature female morphs (andromorph and gynomorph) are observed in natural populations (Fincke et al. 2005). However, in some species male and female body colour also show ontogenetic changes. While in most damselfly species immature individuals spend their maturation period away from the reproductive area (e.g. Miller 1987), in species showing ontogenetic colour changes, immature and mature females often coexist at the reproductive site (e.g. Parr 1973; Fincke 1987). In such species, mate-searching males face the challenge of discriminating mature females from not only mature conspecific males, but also immature males and females. Thus, to more comprehensively understand the challenges males face during mate location, as well as the selective environment under which female coloration has evolved, consideration of the colour phenotype of both mature and immature individuals is necessary. This more complex visual environment raises several questions regarding how female morph coloration may affect male mating decisions. Could differences in colour between immature and mature females facilitate male mate preference for females that carry ripe eggs (i.e. mature females)? How similar is the coloration of “male-like” females to that of mature and immature males? How do differences in colour between mature female morphs correspond to patterns in male mate preference and to observable differences in morph mating frequencies?

Here, for the first time, we explore within and between morph variation in body coloration for the damselfly *Ischnura elegans*, a species for which males and andromorphs are nearly identical to the human eye (i.e. similar in both patterning and coloration). We specifically ask whether colour cues exist that might aid or hamper the ability of males to recognize mature females. We then evaluate whether the colour cues identified using spectrophotometry correspond to male mating interest and realized mating frequencies in the field. We probe two specific questions: (1) are males able to discriminate between immature and mature female morphs?, and (2) do male-like females experience reductions in male mating attention and mating frequency as expected from the male mimicry hypothesis? (see also Cordero Rivera and Sánchez-Guillén 2007; Hammers and Van Gossum 2008; Gosden and Svensson 2009). To address these questions, we combined spectrophotometric measurements with observations of morph mating success in natural populations and experiments in which male mating attempts on live damselfly models were scored.

Materials and methods

Study species

Laboratory cross experiments with *Ischnura elegans* indicate simple Mendelian inheritance patterns, where the proportions of colour morphs in the progeny are consistent with morph type being controlled at an autosomal locus with sex-restricted expression with three alleles with a hierarchy of dominance ($P^{\text{andromorph}} > P^{\text{infuscans}} > P^{\text{rufescens-obsoleta}}$, Sánchez-Guillén et al. 2005). Across their lifetime, male and female *I. elegans* undergo irreversible ontogenetic colour changes (e.g. Hinnekint 1987; Sánchez-Guillén et al. 2005). While ontogenetic colour changes are gradual, we consider seven colour variants, two male and five female, all of which coexist at the reproductive site (e.g. Hinnekint 1987). Male colouration changes gradually from light green to turquoise blue, but for spectral work we limited ourselves to males that were green (immature), and males that were blue (mature) to the human eye. Females start out as one of two morphs: *violacea*, a violet-coloured immature stage and *rufescens* which ranges from pink-orange to brown-ochre body coloration (Cordero et al. 1998). Immature *violacea* develop into one of two mature female morphs: one morph that shows body colouration very similar to the blue male (andromorph) and a morph that shows green body colouration (*infuscans*). While a proportion of females that become mature andromorphs do not pass through the immature *violacea* stage but show light-green immature coloration (similar to immature males) (Sánchez-Guillén et al. 2005), these females are extremely rare in our study populations. Thus, we focus only on the more common *violacea* and *rufescens* immature morphs. Immature *rufescens* develop into mature *rufescens-obsoleta*, which show brown body colouration. *Infuscans* and *rufescens-obsoleta* are both referred to as gynomorph females in that they clearly differ in colour from the male. These two gynomorph female morphs not only differ in colour but also body patterning. *Infuscans* has black humeral stripes which are lacking in *rufescens-obsoleta*, but are found in males and andromorphs. More detailed descriptions and colour drawings of the different morphs can be found in several identification guides (e.g. Askew 2004; Dijkstra and Lewington 2006).

Spectral reflectance

The seven different colour variants described above can be distinguished upon close inspection by the human eye and occur in all our study populations. For this section on colour spectra we focused on male and female morphs of one population only, Walenhoek, Belgium (51° 06′ 30″ N, 4° 21′ 00″ E). While we cannot exclude the possibility that individuals of each morph from different populations may vary slightly in colour, we are interested here in discrete differences between morphs that are in our experience similar across populations. Following capture with an insect net, animals were transported alive in small cages (see Van Gossum et al. 1999) to the laboratory where colour spectra were recorded for the thorax of each individual (June–July 2006 and 2007). Reflectance spectra were obtained with a high-resolution spectrophotometer (Model HR2000, Ocean Optics, Duiven, The Netherlands), at a resolution of 0.3 nm. All reflectance measures were scaled using a white diffuse reflectance standard (WS-1 Diffuse Reflection Standard, Ocean Optics, Duiven, The Netherlands). We used a deuterium-tungsten light source (DH2000-BAL, Ocean Optics, Duiven, The Netherlands), which generates illumination for wavelengths from 200 to 1100 nm. Spectra were taken from the surface of each individual using a coincident reflectance probe (QR 400-7 UV/VIS, Ø 2.6 mm, Ocean Optics, Duiven, The Netherlands) held perpendicular to the surface of the thorax. Because colour measure were taken by holding the probe in hand, it was not possible to measure an identical spot on the thorax twice, thus precluding the estimation of colour score repeatability. Instead, spectra were averaged over 5 independent readings of the lateral area on the left side of the thorax to account for the minor variation in coloration across the colour patch. We re-calibrated our equipment every 2 to 3 measurements using the white standard. All measurements were made at room temperature (22–24°C) and under dark conditions to avoid possible influences of temperature and ambient light on spectrophotometric equipment or colour patterns of the damselflies. We obtained spectra for individuals of all mature and immature morphs of *I. elegans*, including immature ($n = 29$) and mature males ($n = 32$) as well as the three mature female morphs (*infuscans,* $n = 34$; *rufescens-obsoleta,* $n = 35$; andromorph, $n = 27$) and the two major immature female morphs (*rufescens*, $n = 33$; *violacea*, $n = 34$).

As with other odonates (Briscoe and Chittka 2001), damselflies are suggested to perceive colour signals spanning a wavelength range from roughly 300–700 nm (*cf.* Schultz et al. 2008). To address whether damselflies can distinguish female colour morphs knowledge is required regarding both the reflectance properties of each damselfly colour morph, and the visual capabilities of damselflies to perceive and discriminate colour information. In the absence of current information regarding the visual sensitivities of *I. elegans*, we explore colour differences between individuals and morphs using colour parameters that are independent of a particular visual system, but are generally related to visual system responses, namely hue, chroma and intensity. These three colour parameters are commonly used to characterize coloration (Endler 1990). Intensity was defined as the maximum reflectance ($R_{\max}$) at any wavelength between 300 and 700 nm. We defined hue as the wavelength of maximum reflectance ($\lambda_{R\max}$). Chroma was calculated using the following formula:

$$\mathrm{Chroma} = \sum_{\lambda_{R\max}-50}^{\lambda_{R\max}+50} R_i \Bigg/ \sum_{\lambda_{300}}^{\lambda_{700}} R_i$$

This defines chroma as the proportion of total reflected light between 300 and 700 nm that lays 50 nm above and below the wavelength of maximum reflectance (Shawkey et al. 2003).

For each of the three colour parameters, we compared all colour morphs using one-way ANOVAs in Systat 10 (Systat Software, Inc., Richmond, CA). Normality and equal variance of data were verified prior to running statistical tests. For multiple comparisons, Bonferroni correction was used to maintain an experiment-wide α of 0.05. We report central tendency in the text and tables as least squares means ± standard error.

Morphs, mating success and male mate preference

Sexual interactions between males and females were studied using two approaches. First, we observed proportions of mated versus unmated individuals in wild populations that varied in relative morph frequencies. Such mating probabilities were determined for all female ontogenetic and colour morphs and can be seen as the outcome of conspecific interactions in the field, including both intra- and intersexual interactions. Second, we presented live individuals to males in experimental trials, allowing experimental evaluation of male mating preferences for different colour morphs. This experiment was only conducted with sexually mature individuals. While for the first approach, results may depend on both male and female behaviour, female behaviour was largely excluded in the second approach. Study populations were all located in the Netherlands at locations >5 km apart and each associated with different water bodies. Observations and experiments were conducted from June to August 2008, during hours of damselfly reproductive activity (9 am to 3 pm local time) when weather conditions were favourable (i.e. individuals were seen mating).

For the first approach we examined how many individuals of each female morph were mated versus unmated. When single, females were scored as unmated, in contrast to mated females that were attached to a male (either in tandem—a pre-copula stage in damselflies—or in copula). Mating probabilities were determined in each study population ($N = 9$) by walking through the vegetation and randomly catching all observed individuals one by one (when single) or per two (when mated). For each individual we noted gender, ontogenetic stage, and colour morph. Before release, animals were marked with two dots on the right forewing to preclude multiple counts. To avoid potential sampling or observer bias, all vegetation types present at the water's edge were haphazardly sampled and all sampling was conducted by the same person (JVH). We calculated the absolute mating probability for each morph by dividing the number of mated individuals by the total number observed for that morph. Data were analysed using generalized linear models with binomial error structure and logit-link function in SAS, version 9.1 (SAS Institute, Inc.). Morph was treated as fixed factor and population was included as a random factor.

For the second approach, we presented mature live models (andromorph, *infuscans*, *rufescens-obsoleta* and males) to focal males (for methodology, see also Hammers et al. 2009; Ting et al. 2009 and references therein) during the summer of 2008. These live models were glued (Uhu Power glue) with the anterior part of the ventral side of the thorax and the mandibles attached to the end of a thin wooden stick. In this way, possible behavioural differences between sex and morphs, such as differences in the use of microhabitat, are excluded. Models were presented to mature males by holding the stick at a 40°–70° angle and <1 body length away from the focal male. The four models (andromorph, *infuscans*, *rufescens-obsoleta* and males) were alternately presented to a different (unique) focal male to reduce possible bias in male response due to any subtle temporal variation in male reproductive activity. A single trial was completed when each of the models had been presented, in a systematic and randomised order, to six different focal males. Thus for a single trial, a total of 24 unique focal males were used (4 models presented to 6 different males each). In total, 15 trials were conducted at each study population (i.e. 15 trials, 4 models, 6 presentations per model = 360 preference tests with unique males per population). For each model, we compared how many of the six focal males showed mating interest (see next paragraph for a description on how we classified sexually interested versus non-interested male reactions). The attractiveness of each model was thus scored as the proportion of six males who showed interest (i.e., a maximum score of 6 out of 6 males). Consequently, the model is the analytical unit rather than the focal male, thereby avoiding pseudo-replication. Once each model had been presented to six males, the model was immediately euthanized by immersion in ethanol.

Presentations were done at four populations selected for their contrasting andromorph frequencies. Andromorph frequency was estimated prior to the presentation experiments by dividing the total number of mature andromorphs by the total number of mature females caught in multiple sweeps with an insect net. Two populations had an andromorph frequency >60% (Koudekerke: 81.8% and Maasvlakte: 64.6%), while the other two populations had andromorph frequency <40% (Goudriaan: 38.2% and Kekerdom, 25.8%). Apart from variation in andromorph frequencies, the four populations were similar, i.e. large numbers of animals and habitats showing comparable variation in vegetation cover (e.g. grasslands, reed beds, sandbanks, and shrubs). Because earlier work showed that andromorph *I. elegans* use more open habitat while gynomorphs occur more in dense habitat (Van Gossum et al. 2001a), we specifically addressed variation in vegetation cover in the experiment in the following way. From the six presentations made with each model, three presentations involved males in open habitat (i.e. low grassland) and three involved males in dense habitat (i.e. reed).

Male responses to models were categorised as either a tandem attempt or non-tandem attempt. Non-tandem attempts included: (1) no reaction at all—the observation was ended after 120 s; (2) the focal male showed no interest and flew away; or (3) the focal male approached and made contact with the stick or the model, but without showing sexual interest. In contrast, a tandem attempt indicates sexual interest by male damselflies. In such an event, the male makes contact with the model and attempts to grab the model with his anal appendages (e.g. Corbet 1999). Focal males were caught after the experiment and given a mark on the wing before release, avoiding multiple presentations to the same focal male. In only a few occasions, e.g. when the male escaped into dense vegetation, we did not succeed in catching the focal male, and thus we think it unlikely that males participated more than once in our experimental trials.

When comparing patterns of male mate preference between vegetation types, no differences were observed in any of the four study populations (all $P > 0.2$). Hence for subsequent analyses, vegetation types were pooled (proportion of tandem attempts towards each model calculated based on six instead of three presentations to different males). Variation in male mate preferences between models and across populations were analysed using generalized linear mixed models with binomial error distribution and link logit function in SAS, version 9.2 (SAS Institute Inc. 2005). Model type and population (both categorical) and their interaction were included as fixed factors. To account for inter-trial differences (e.g. due to varying weather conditions), trial was added as random factor (random intercept and slope, unstructured covariance) to the statistical model. Degrees of freedom of the fixed effects F-tests were adjusted for statistical dependence using the Kenward and Roger (1997) method. In addition, this analysis was repeated for all populations separately because a significant type*population-interaction was detected (see below). Model selection was started with the full model, and then preceded with the stepwise removal of non-significant terms. The non-significant factor with the highest *P*-value was first removed and the final model was obtained when only significant terms remained (Verbeke and Molenberghs 1997).

Results

Spectral analyses

Male and female morphs differ from each other spectrally in a number of different ways (Fig. 1), captured by statistical differences in the spectral parameters hue, chroma and intensity (Fig. 2, Table 1). Several features obvious in the spectral plots (Fig. 1) help with interpretation of the patterns seen in the spectral parameters. First, all immature morphs, including the immature male morph, exhibit an additional spectral reflectance peak in shorter wavelengths that is not present in mature morphs of either sex (Fig. 1). This additional short-wavelength spectral peak is responsible for lowering the chroma of all

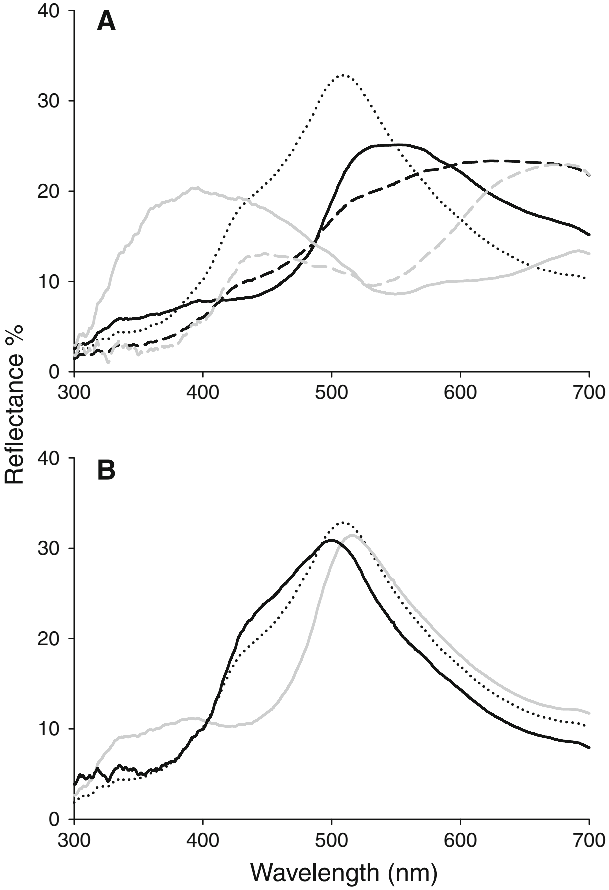

Fig. 1 Spectral averages for all female (**a**) and male (**b**) colour morphs. For both sexes, immature morph spectra are in grey and mature morph spectra are in black. Female morphs represented are *infuscans* (*solid black line*), *rufescens-obsoleta* (*dashed black line*), andromorph (*dotted black line*), *violacea* (*solid grey line*) and *rufescens* (*dashed grey line*). The andromorph female is also plotted in B to allow for easier comparison between this putative male mimic and its two potential colour models

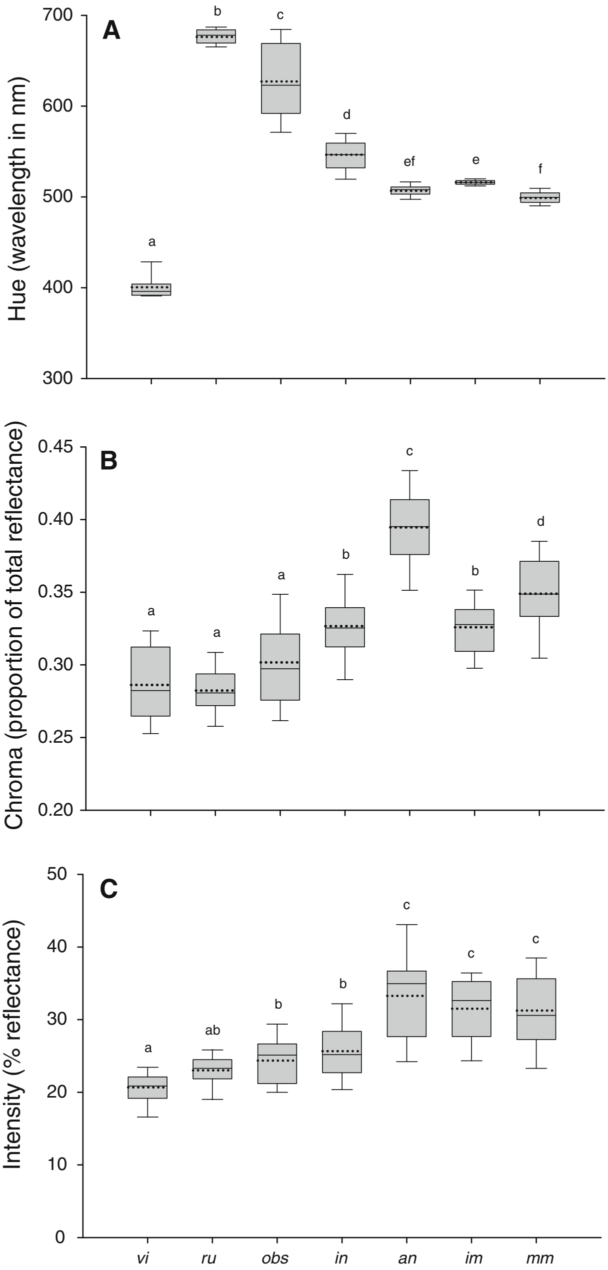

Fig. 2 Boxplots showing comparison across morphs for hue (**a**), chroma (**b**) and intensity (**c**). Boxes labelled with different lowercase letters are statistically distinct from each other. *Dotted lines* within boxes are means, *solid lines* are medians. *Upper* and *lower box* sides represent 75% confidence intervals and *error bars* represent 95% confidence intervals. *vi* = *violacea*; *ru* = *rufescens*; *ro* = *rufescens-obsoleta*; *in* = *infuscans*; *an* = andromorph; *im* = immature male; *mm* = mature male

Table 1 Least-squares means ± standard errors for hue, chroma and intensity, as well as statistical results from one-way ANOVAs comparing morphs

Morph	*n*	Hue ± SE (nm)	Statistical groups	Chroma ± SE	Statistical groups	Intensity ± SE (% reflectance)	Statistical groups
Violacea (immature)	34	400.50 ± 3.35	a	0.286 ± 0.005	a	20.68 ± 0.79	a
Rufescens (immature)	33	676.15 ± 3.40	b	0.282 ± 0.005	a	23.02 ± 0.81	ab
Rufescens-obsoleta	35	627.14 ± 3.30	c	0.302 ± 0.005	a	24.36 ± 0.78	b
Infuscans	34	546.40 ± 3.35	d	0.327 ± 0.005	b	25.66 ± 0.79	b
Andromorph	27	506.70 ± 3.76	ef	0.395 ± 0.005	c	33.27 ± 0.89	c
Immature male	29	516.03 ± 3.63	e	0.326 ± 0.005	b	31.50 ± 0.86	c
Mature male	32	498.81 ± 3.45	f	0.349 ± 0.005	d	31.26 ± 0.82	c

The column ‘statistical groups’ refers to which of the morphs significantly do differ for hue, chroma and intensity: when same letters apply there is no significant differences

immature morphs below that of mature morphs of the same sex. In the *violacea* female immature morph, this peak in the shorter wavelengths is so pronounced that it dominates the spectral reflectance, resulting in the violet appearance to human observers for which the morph is named (Table 1).

Second, with the exception of the andromorph female, all morphs differ significantly from each other in hue, which we (and likely damselfly conspecifics) perceive as the predominant colour of each morph. Further, the andromorph does not differ statistically from either the mature or immature male morph in hue, although the male morphs differ from each other (Table 1).

Several other differences are worth noting as well. First, the andromorph coloration is both the brightest (highest peak reflectance) and most chromatic of any morph. For the latter parameter (chroma), andromorphs have significantly higher values than all other morphs, whereas for the former parameter (intensity), andromorphs have the highest values on average, but do not differ significantly from the two male morphs (Fig. 2). Additionally, males and andromorph*s* have significantly brighter colour patterns than all other morphs.

Morphs, mating success and male mate preference

Female morph frequencies varied across the nine populations studied, with immature females constituting from 13 to 43% (28% ± 3% SE) of the total number of females and andromorph females making up 31 to 61% (43% ± 3% SE) of the mature female population (see Table 2, the andromorph frequency was determined by dividing the total number of andromorphs by the total number of mature females). Female morphs differed in mating frequencies ($\chi^2 = 344.75$, $df = 4$, $P < 0.001$; Fig. 3). Specifically, immature females were found mated much less frequently compared to mature females. Furthermore, andromorphs were found mated consistently less often compared to *infuscans* and *rufescens-obsoleta* females whereas the two gynomorphs did not differ in their mating frequency (Fig. 3).

Table 2 Number of females of the different ontogenetic and colour morphs of the damselfly *Ischnura elegans* in our nine study populations: column 2–6 refers to the total number of individuals per morph, columns 7–11 refers to the individuals observed mating of the total; *vi* = *violacea*, *ru* = *rufescens*, *an* = andromorph, *in* = *infuscans* and *ro* = *rufescens-obsoleta*

Study site (latitude N, longitude E)	Total number of females in population					Number of mated females in population				
	vi	ru	an	in	ro	vi	ru	an	in	ro
Maasvlakte (51°56′26″, 4°01′29″)	30	51	53	21	33	0	6	16	7	15
Koudekerke (51°29′09″, 3°32′25″)	57	37	93	26	34	2	4	55	20	25
Kromme Rade (52° 12′ 93″, 5°5′ 07″)	23	14	35	24	20	3	0	19	20	13
Zouweboezem (51°57′05″, 4°59′55″)	17	32	44	33	63	1	3	25	32	59
Annabos (51°51′29″, 4°05′31″)	12	26	48	24	42	3	1	26	17	28
Tenellaplas (51°54′04″, 4°04′06″)	13	13	58	20	54	1	2	38	19	50
Slingeland (51°53′06″, 4°53′58″)	10	16	25	22	22	0	2	10	19	17
Poelbos (51°29′40″, 3°51′28″)	14	17	38	21	20	0	3	20	20	17
Millingerwaard (51°52′12″, 6°0′ 25″)	6	8	30	30	33	0	1	23	28	27

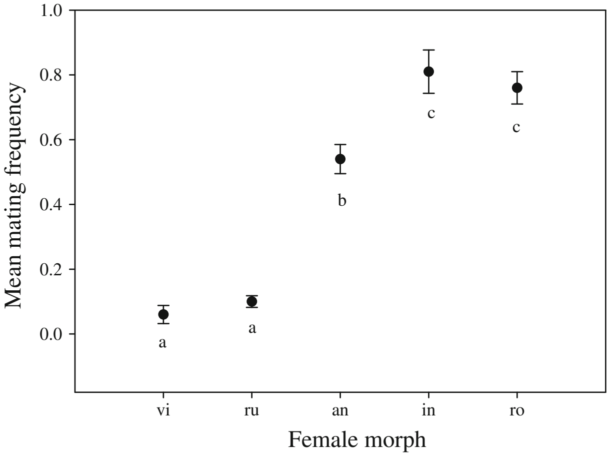

Fig. 3 Mean mating frequencies (the number of mated individuals of a morph versus the total number of individuals of that morph in the population) of the different ontogenetic and colour morphs of the damselfly *Ischnura elegans* ($N = 9$ populations): *vi* = *violacea*; *ru* = *rufescens*; *ro* = *rufescens-obsoleta*; *in* = *infuscans*; *an* = andromorph. Different lowercase letters indicate significant statistical differences ($P < 0.05$) in the mean proportion of tandem attempts among models based on Tukey HSD

For the male mate preference experiments, a significant morph*site interaction was observed, indicating that differences in male behavioural responses to morphs varied between study populations ($F_{9,\ 224} = 2.60$, $P = 0.007$; Fig. 4). Therefore, analyses were conducted separately for each study population. In Koudekerke ($F_{3,\ 47.5} = 4.72$, $P = 0.006$), Maasvlakte ($F_{3,\ 46.6} = 6.42$, $P = 0.001$) and Goudriaan ($F_{3,\ 47.4} = 3.94$, $P = 0.014$), model types differed in the proportion of tandem attempts they elicited. In all three populations, the proportion of tandem attempts did not differ between andromorphs and males, and both models were least preferred by males (Fig. 4). In contrast, *infuscans*

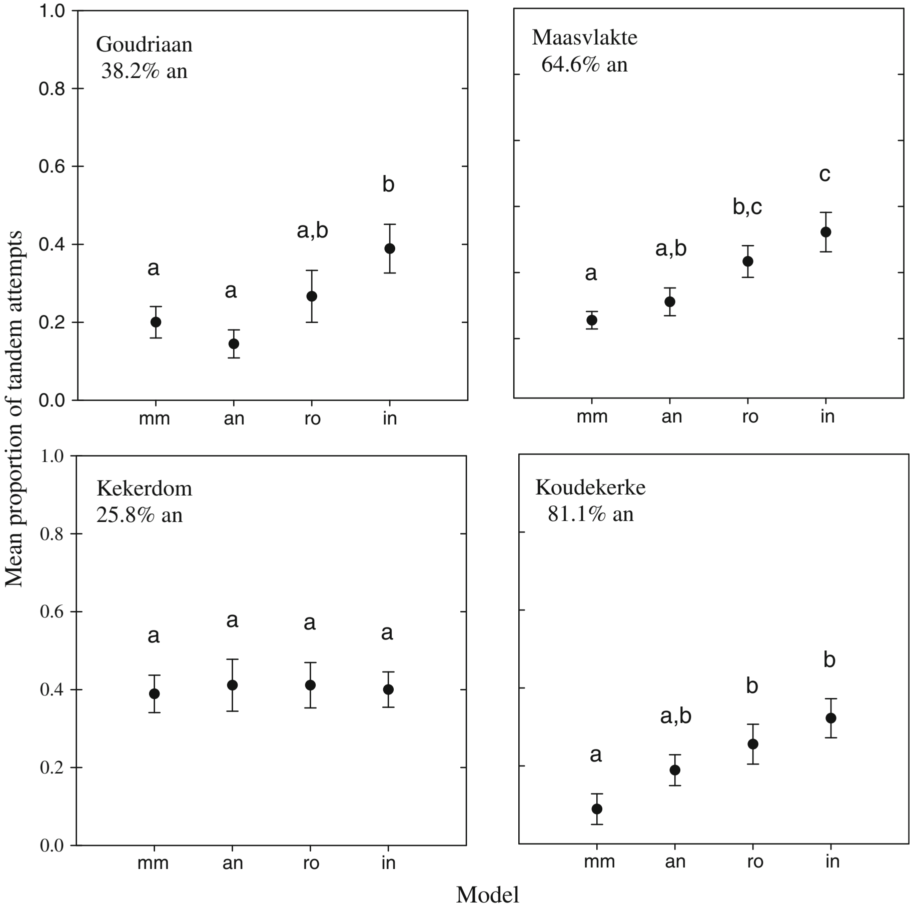

Fig. 4 Male mate preference for males and mature female colour morphs of the damselfly *Ischnura elegans* in four study populations; *mm* = mature male; *ro* = *rufescens-obsoleta*; *in* = *infuscans*; *an* = andromorph. Different lowercase letters indicate significant statistical differences ($P < 0.05$) in the mean proportion of tandem attempts among models based on Tukey HSD for within site analyses

females were highly preferred by males with the proportion of tandem attempts being significantly higher than that on andromorphs, except in Koudekerke. Furthermore, mate searching males showed an intermediate interest for *rufescens-obsoleta*; male tandem attempts with this morph were not statistically different from either the andromorph or *infuscans* (Fig. 4). In the fourth population (Kekerdom), however, all model types were equally attractive to males ($F_{3,56} = 0.04$, $P = 0.988$). Also, the mean proportion of tandem attempts in this population was relatively high (±0.4, see Fig. 4).

Discussion

The results of our spectral analyses suggest that a number of cues relevant to colour perception are available for behavioural decisions by mate-searching males and may help in understanding morph mating frequencies in the wild. First, the unimodal versus bimodal spectra identified by our spectral measurements may allow mate-searching males to distinguish mature from immature individuals. This possibility is supported by field observations that immature females are found in copula much less frequently as compared to mature females (Fig. 1; see also Hammers et al. 2009). From an evolutionary point of view, such apparent discrimination between mature and immature prospective mates makes intuitive sense. Indeed, male attempts to mate with immature females may render little immediate fitness benefits, as these females may not have mature eggs yet and last sperm precedence will reduce the likelihood of fertilisation when females mate multiply, as is the case in *I. elegans* (Cooper et al. 1996). Thus for males, it may be beneficial to spend time and energy attempting to locate mature females rather than mating with immature females. From the female perspective, immature females may reject male mating attempts until they carry a clutch of ripe eggs (Banham 1990; Fincke 1997). Thus, low mating frequencies of immature females may be due to female avoidance behaviour and/or due to the ability of males to identify and avoid matings with immature females. The fact that immature morphs show an additional spectral peak (see Fig. 1) which results in lower chroma values, suggests that immature morphs of both sexes are readily distinguishable from their mature conspecifics. Also, odonates are known to be highly visual and colour sensitive, having up to five spectral types of photoreceptors (e.g. the pentachromatic odonate *Hemicordulia tau* with peak sensitivities at 330, 410, 460, 525 and 630 nm, Yang and Osorio 1991). If spectral abilities of male *I. elegans* are comparable to those of other odonates, the uni- and bimodal spectra of immature and mature females should be readily perceivable by mate-searching males (see also Fig. 2).

We would further expect males to be able to distinguish gynomorphs from immature and mature males using differences in hue, chroma and intensity. Andromorphs, however exhibit colour patterns that are statistically indistinguishable from that of males. It is intriguing that while immature and mature males can be (statistically) distinguished, andromorphs cannot be distinguished statistically from either ontogenetic male morph, suggesting that andromorph coloration may represent an approximation of a generalized male phenotype which includes the appearance of immature males. In any event, our analyses suggest that andromorph *I. elegans* coloration is so similar to conspecific males with respect to spectral information that a male would likely need to rely on other cues, such as behaviour, to recognize these females as potential mates. This finding stands in contrast to previous work in species where andromorphs and males are statistically distinguishable (Fincke et al. 2007) and thus perhaps more readily discriminated by mate-searching males.

In line with our spectral results, we detected that experimental and field mating probabilities are not simple reflections of female morph frequencies, indicating that males do not mate randomly with female morphs. Indeed, andromorphs appear to mate less frequently compared to both gynomorph females in all nine field populations, even when they are the most common mature female morph (see also Cordero Rivera and Sánchez-Guillén 2007; Hammers and Van Gossum 2008; Gosden and Svensson 2009 for similar observations). Also, our male mate preference experiments show that andromorphs are the least attractive female morph and that males fail to distinguish between male conspecifics and andromorphs in experimental trials. One exception to this pattern occurred in the Kekerdom population, where males did not appear to distinguish between female morphs and even appeared to be unable to distinguish between the sexes. This discrepancy may be explained from the fact that this population was studied towards the end of the reproductive season of *I. elegans* after a period of bad weather. We suggest that males under these conditions may have been desperately searching for mating opportunities and no longer discriminated among morphs or even sexes. Furthermore, it is of interest that previous studies examining female mate avoidance tactics have shown that andromorphs behave more male-like than gynomorphs in that they confront and chase mate-searching males (e.g. Van Gossum et al. 2001b).

Together, present and previous findings on female morph mating frequencies, behaviour and reflectance spectra appear consistent with the hypothesis that andromorphs engage in functional male-mimicry to avoid excessive sexual harassment by interfering with the ability of males to recognize them as potential partners (cf. Robertson 1985; Sherratt 2001). However, this does not exclude a possible role of negative frequency-dependent selection in the maintenance of female polymorphism, i.e. where the most common female morph receives the most male attention but experiences harassment-related reductions in realized fecundity compared to the less common morph (see Van Gossum et al. 2001a, b; Fincke 2004; Svensson et al. 2005). For example, recent work with the female polymorphic damselfly *Nehalennia irene* showed results consistent with both male mimicry and negative frequency-dependent selection suggesting that they are not mutually exclusive and can occur simultaneously (Ting et al. 2009). On the other hand, if andromorphs mate less often they may sometimes not copulate at all as observed in a field study on *I. elegans* (Cordero et al. 1998). Thus, while gynomorphs may suffer reductions in realized fecundity from excessive male harassment, andromorph fitness may be constrained by difficulties in securing even a single copulation.

Selection on female colour morphs may not be limited to sexual interactions with males. It has been suggested that andromorphs are more vulnerable to predation because of their conspicuous body coloration (e.g. Robertson 1985). In addition, several other factors may be important, like differences in morph behaviour (e.g. gynomorphs may be more likely end up in spider webs than andromorphs (Van Gossum et al. 2004)) and different responses of female morphs to ambient temperature (e.g. Bots et al. 2009). More work is clearly needed to unravel the complex interactions that have shaped and continue to maintain female colour polymorphism in this group (see also Cordero et al. 1998; Van Gossum et al. 2008; Svensson et al. 2009 for more detailed discussions on this).

Acknowledgments We much thank Christopher Beatty, Adolfo Cordero Rivera, Arne Iserbyt, Luc De Bruyn, Ronald Rutowski, Thomas Sherratt, Tom Van Dooren and Stefan Van Dongen for useful discussion and suggestions on previous versions of our manuscript. We further thank Luc De Bruyn for help in performing analyses. HVG is a postdoctoral fellow with the Fund for Scientific Research—Flanders (FWO-Flanders). Our work benefited from a research grant from the University of Antwerp to HVG. All observations and experiments complied with the current laws and ethical guidelines for The Netherlands.

References

Arnqvist G, Rowe L (2005) Sexual conflict. Princeton University Press, Princeton

Askew RR (2004) The dragonflies of Europe. Harley Books, Essex

Banham WMT (1990) Non-random mating in the polymorphic damselfly *Ischnura elegans*. Ph.D. Thesis, University of Manchester

Bond AB, Kamil AC (2002) Visual predators select for crypticity and polymorphism in virtual prey. Nature 415:609–613

Bots J, De Bruyn L, Van Dongen S, Smolders R, Van Gossum H (2009) Female polymorphism, condition differences, and variation in male harassment and ambient temperature. Biol J Linn Soc 97:545–554

Briscoe AD, Chittka L (2001) The evolution of color vision in insects. Annu Rev Entomol 46:471–510

Contreras-Garduno J, Buzatto BA, Serrano-Meneses MA, Najera-Cordero K, Cordoba-Aguilar A (2008) The size of the red wing spot of the American rubyspot as a heightened condition-dependent ornament. Behav Ecol 19:724–732

Cooper G, Miller PL, Holland PWH (1996) Molecular genetic analysis of sperm competition in the damselfly *Ischnura elegans* (Vander Linden). Proc R Soc B 263:1343–1349

Corbet PS (1999) Dragonflies: behaviour and ecology of Odonata. Martins, Harley Books, Essex

Cordero Rivera A, Sánchez-Guillén RA (2007) Male-like females of a damselfly are not preferred by males even if they are the majority morph. Anim Behav 74:247–252

Cordero A, Santolamazza Carbone S, Utzeri C (1998) Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans* (Odonata). Anim Behav 55:185–197

Dijkstra KD, Lewington R (2006) Field guide to the dragonflies of Britain and Europe. British Wildlife Publishing, Dorset

Edmunds M (2000) Why are there good and poor mimics? Biol J Linn Soc 70:459–466

Endler JA (1990) On the measurement and classification of colour in studies of animal colour patterns. Biol J Linn Soc 41:315–352

Fincke OM (1987) Female monogamy in the damselfly *Ischnura verticalis* Say (Zygoptera: Coenagrionidae). Odonatologica 16:129–143

Fincke OM (1997) Conflict resolution in the Odonata: implications for understanding female mating patterns and female choice. Biol J Linn Soc 60:201–220

Fincke OM (2004) Polymorphic signals of harassed female odonates and the males that learn them support a novel frequency-dependent model. Anim Behav 67:833–845

Fincke OM, Jödicke R, Paulson D, Schultz DT (2005) The evolution and frequency of female color morphs in Holarctic Odonata: why are male-like females typically the minority. Int J Odonatol 8:183–212

Fincke OM, Fargevieille A, Schultz TD (2007) Lack of innate preference for morph and species identity in mate-searching *Enallagma* damselflies. Behav Ecol Sociobiol 61:1121–1131

Gosden TP, Svensson EI (2007) Female sexual polymorphism and fecundity consequences of male mating harassment in the wild. PLoSONE 2(6):e580

Gosden TP, Svensson EI (2009) Density-dependent male mating harassment, female resistance, and male mimicry. Am Nat 173:709–721

Gross MR (1996) Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol Evol 11:92–98

Hammers M, Van Gossum H (2008) Variation in female morph frequencies and morph mating frequencies: random, frequency-dependent harassment or male-mimicry? Anim Behav 76:1403–1410

Hammers M, Sánchez-Guillén RA, Van Gossum H (2009) Differences in mating propensity between immature female colour morphs in the damselfly *Ischnura elegans* (Insecta: Odonata). J Insect Behav 22:324–337

Hardling R, Bergsten J (2006) Nonrandom mating preserves intrasexual polymorphism and stops population differentiation in sexual conflict. Am Nat 167:401–409

Hinnekint BON (1987) Population dynamics of *Ischnura e. elegans* (Vander Linden) (Insecta: Odonata) with special reference to morphological colour changes, female polymorphisms, multi-annual cycles, and their influence on behaviour. Hydrobiologia 146:3–31

Houde A (1997) Sex, color and mate choice in guppies. Princeton University Press, Princeton

Joop G, Siva-Jothy MT, Rolff J (2006) Female colour polymorphism: gender and the eye of the beholder in damselflies. Evol Ecol 20:259–270

Jormalainen V, Merilaita S, Riihimaki J (2001) Cost of intersexual conflict in the isopod *Idotea baltica.* J Evol Biol 14:763–772

Kenward MG, Roger JH (1997) Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53:983–997

Miller PL (1987) Dragonflies, 1st edn. Cambridge University Press, Cambridge

Miller MN, Fincke OM (1999) Cues for mate recognition and the effect of prior experience on mate recognition in *Enallagma* damselflies. J Insect Behav 12:801–814

Mundy NI, Badcock NS, Hart T, Scribner K, Janssen K, Nadeau NJ (2004) Conserved genetic basis of a quantitative plumage trait involved in mate choice. Science 303:1870–1873

Oliveira RF, Taborsky M, Brockmann HJ (2008) Alternative reproductive tactics. Cambridge University Press, Cambridge

Parr MJ (1973) Ecological studies of *Ischnura elegans* (Vander Linden) (Zygoptera: Coenagrionidae). Survivorship, local movements and dispersal. Odonatologica 2:159–174

Plath M, Parzefall J, Schlupp I (2003) The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). Behav Ecol Sociobiol 54:303–309

Robertson HM (1985) Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburi*: females mimicking males. Anim Behav 33:805–809

Sánchez-Guillén RA, Van Gossum H, Cordero Rivera A (2005) Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae). Biol J Linn Soc 85:471–481

SAS Institute Inc (2005) The Glimmix procedure. SAS/STAT Users Guide, Version 9.1, Cary, NC

Schultz TD, Anderson CN, Symes SB (2008) The conspicuousness of colour cues in male pond damselflies depends on ambient light and visual system. Anim Behav 76:1357–1364

Seehausen O, Mayhew PJ, Van Alphen JJM (1999) Evolution of colour patterns in East African cichlid fish. J Evol Biol 12:514–534

Shawkey MD, Estes AM, Siefferman LM, Hill GE (2003) Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colours. Proc R Soc B 270:1455–1460

Sherratt TN (2001) The evolution of female-limited polymorphisms in damselflies: a signal detection model. Ecol Lett 4:22–29

Sherratt TN (2002) The evolution of imperfect mimicry. Behav Ecol 13:821–826

Svensson EI, Abbott J, Härdling R (2005) Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. Am Nat 165:567–576

Svensson EI, Abbott JK, Gosden TP, Coreau A (2009) Female polymorphisms, sexual conflict and limits to speciation in animals. Evol Ecol 23:93–108

Ting J, Bots J, Perez Jvostov F, Van Gossum H, Sherratt T (2009) Effects of extreme variation in female morph frequencies on the mating behaviour of male damselflies. Behav Ecol Sociobiol (in press)

Van Gossum H, Stoks R, Matthysen E, Valck F, De Bruyn L (1999) Male choice for female colour morphs in *Ischnura elegans* (Odonata, Coenagrionidae): testing the hypothesis. Anim Behav 57:1229–1232

Van Gossum H, Stoks R, De Bruyn L (2001a) Reversible frequency-dependent switches in male mate choice. Proc R Soc B 268:83–85

Van Gossum H, Stoks R, De Bruyn L (2001b) Frequency-dependent male mate harassment and intraspecific variation in its avoidance by females of the damselfly *Ischnura elegans*. Behav Ecol Sociobiol 51:69–75

Van Gossum H, Adriaens T, Dumont H, Stoks R (2004) Sex- and morph-specific predation risk: colour or behaviour as an explanation? Eur J Entomol 101:373–377

Van Gossum H, Sherratt TN, Cordero Rivera A (2008) The evolution of sex-limited colour polymorphisms. In: Córdoba-Aguilar A (ed) Dragonflies: model organisms for ecological and evolutionary research. Oxford University Press, Oxford, pp 219–229

Verbeke G, Molenberghs G (1997) Linear mixed models. A SAS-oriented approach. Springer, New York

Yang EC, Osorio D (1991) Spectral sensitivities of photoreceptors and lamina monopolar cells in the dragonfly *Hemicordulia tau*. J Comp Physiol A 169:663–669